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Specialty Corns

Edited by
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Preface

Corn is one of the major cereal grains grown in the world, exceeded only by rice and wheat in terms of quantity produced. In the U.S., corn is produced on 70 to 80 million acres annually and plays an important role in the economy of the country. Hybrid corn was introduced in the U.S. in the 1930s, and average production per unit area increased from 35 bushels per acre for the period of 1945 to 1950 to more than 120 bushels per acre for the decade of the 1990s. In 1998, corn growers in the U.S. produced 9.77 billion bushels with an average yield of 134 bushels per acre. Nearly 60% of the increase in yield was because of genetic improvement of hybrids.

Over 95% of the corn produced in the U.S. is marketed as commodity corn, which is primarily yellow dent corn. Commodity corn is fed to livestock (55%), is exported (29%) and largely used for feed, is used in wet milling (12%), and is used in dry milling (4%). Although a typical supermarket displays over 1200 items that include one or more ingredients from corn, less than 5% of the total U.S. corn production is marketed as specialty corns. Several distinct types of specialty corns are available (popcorn, sweet corn, high-protein quality corn, high waxy corn, high oil corn, etc.), but individually each has less than 1% of the total corn market. Alternative uses of the corn that can be produced in large quantities in the U.S. would have broad implications in the economy of the nation and for the individual grower.

Greater emphasis is being given to the development of new processes and new products to enhance the value of corn. One new use for corn in recent years has been the extraction of ethanol for use as corn-based fuels for cars and trucks. About 600 million bushels of corn in the U.S. are converted to ethanol each year, adding at least 30 cents greater return per bushel of corn and greater returns for the producer.

Development of superior specialty corns that have consistent performance in quality and in quantity has not received the same emphasis in genetics and breeding that has been given to the yellow dent corns. Each specialty corn has traits that require special emphasis, but the basic germplasm and breeding methods used to improve yellow dent corn often are used in development of improved specialty corns. However, because of the standards required for specialty corns, methods of development and improvement are usually more complex than those for yellow dent commodity corn. The same standards of performance are desired, but the genetics of the specialty traits often are in conflict with the standards used for yellow dent corns.

Specialty corn programs have unique characteristics that require careful handling and monitoring during their development for specific needs. The objective of this volume is to provide a summary of the germplasm, methods of development, and specific problems involved for some specialty corns. Chapters on development of blue corn and baby corn are new to the Second Edition. Although blue corn and baby corn have specific niche markets, they are becoming more recognized in the human diets, especially for specific ethnic groups. Two chapters provide an introduction of the kernel mutants and of the different types of starch modifications available in corn to illustrate the variation in properties of the corn kernel. Eight chapters provide detailed descriptions of the germplasm and methods used to develop value-added corns that are related to food and feed uses. One chapter summarizes the processes used for food products derived from corn. The last three chapters describe methods used to develop corn for manufacture of pipes, corn for silage, and corns for temperate areas. Some traditional markets of yellow dent corn other than for feed or for export — ethanol, corn sweeteners, and corn starches — were not addressed in this volume. Most chapters have been revised to include the latest information and more recent references; exceptions were the chapters on high amylose and waxy corn and breeding white

endosperm corn. Only minor revisions were included in the chapter on high quality protein corn. It is our desire that the information provided will serve as a guide and reference to those engaged in development of specialty corns and to those that are considering the possibilities of initiating specialty corn programs. U.S. corn producers have become extremely proficient at producing more grain per unit area, but profit margins continue to decrease. Adding value either via alternative products from the large volumes of grain produced or development of specialty corns is of interest to producers and processors. We hope the revised edition can enhance the future uses of corn.

I wish to thank the contributing authors for their tireless efforts, their cooperation in preparing and revising manuscripts, and their patience during the completion of the volume.

Arnel R. Hallauer

Editor

Arnel R. Hallauer is a C. F. Curtiss Distinguished Professor in Agriculture with the Department of Agronomy, College of Agriculture at Iowa State University. He is a native of Kansas who graduated from Kansas State University in 1954 with a B.S. degree in plant science. After serving with the U.S. Army (1954–1956), he enrolled at Iowa State University where he fulfilled the requirements for M.S. (1958) and Ph.D. (1960) degrees in plant breeding. His professional career started in 1958 as an agronomist with the U.S. Department of Agriculture breeding for resistance to the European corn borer. During 1961–1962, Hallauer was stationed in the Genetics Department at North Carolina State University to study the inheritance of quantitative traits. In 1962, he returned to Iowa, where his research interests focused on the inheritance of quantitative traits, selection methods for the enhancement of germplasm resources, effectiveness of methods for evaluating inbred lines for their potential in hybrids, and evaluation, adaptation, and enhancement of exotic germplasm sources for use in temperate area breeding programs. Hallauer retired from the U.S. Department of Agriculture in 1989 and accepted a position in corn breeding at Iowa State University, where he continues to pursue research on breeding methods and germplasm enhancement. He teaches graduate-level courses in plant breeding. He has been the major advisor for more than 80 students who have fulfilled the requirements for M.S. and Ph.D. degrees with majors in plant breeding, and he has served on the program of study committees for more than 145 graduate students.

Hallauer has been recognized for his basic research in the application of quantitative genetic theory to applied plant breeding for line and hybrid development and germplasm enhancement. A compilation of the quantitative genetic studies conducted in corn and their applications to corn breeding were summarized and published in *Quantitative Genetics in Maize Breeding*. The book has become widely used by breeders of corn and other crop species in the U.S. and internationally. He has been recognized for his contributions with election to the National Academy of Sciences in 1989 and inclusion in the U.S. Department of Agriculture's Agricultural Research Science Hall of Fame in 1992. He received the Iowa Governor's Science Medal in 1990 and the National Council Commercial Plant Breeders/Genetics and Breeding Award in 1984.

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1 Kernel Mutants of Corn

Charles D. Boyer and L. Curtis Hannah

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I. INTRODUCTION

Corn is the major cereal crop of the U.S. and the second most important cereal crop worldwide. The diversity of potential uses for corn was realized by the Native Americans prior to the first voyage of Columbus. Today, corn production in the U.S. provides feed, food, and a resource for many unique industrial and commercial products. The potential to enhance use of corn for specialty purposes based on existing uses, and develop new products to meet the needs of future generations, provides the plant breeder/plant scientist with unique challenges. The grain of corn is composed of several chemicals of commercial value. The mature kernel is composed of 70 to 75% starch, 8 to 10% protein, and 4 to 5% oil.[1] The two major structures of the kernel, the endosperm and the germ (embryo), constitute approximately 80 and 10% of the mature kernel dry weight, respectively. The endosperm is largely starch (approaching 90%) and the germ contains high levels of oil (30%) and protein (18%). The oil and protein often are of commercial value as by-products from the production of starch. Immature kernels contain relatively high levels of sugars and lesser amounts of starch, protein, and oil, which accumulate during development.[2] The sugar content is the most clearly recognizable component of sweet corn quality as sweet corn is eaten at an immature stage of development.[3] By utilizing genetic variation, the composition of the kernel can be changed for both the quantity and quality (structure and chemical diversity) of starch, protein, and oil throughout kernel development. The ability of future generations of scientists to utilize existing genetic variation and to create new avenues to designing novel variation in corn composition will provide the basis for the development of the next generation of specialty corns.

In this chapter we will review the well-developed body of information on the genetic variation known to affect the composition, development, and structure of corn kernels. As part of this discussion we will provide some practical suggestions on the construction of various genotypes. With the increased interest in the potential of novel corns for special purposes,[4–11] we believe these suggestions will be useful to biochemists, food scientists, plant scientists, and others beginning to use various genes and gene combinations in their work. In addition, we will provide a review of the relationship of the genetic variation to the modification of metabolism in the kernel. We feel the future expansion of this understanding of metabolism will provide the basis for the development of corns designed for specific uses. Finally, we will provide a brief description of the potential that the new technologies of genetic engineering provide for the creation of new variation in the future.

The importance of corn as a commodity has led to a large number of reviews and books devoted to the nature of corn. Some of the previous reviews the reader may wish to consult include reviews of corn genetics,[12] carbohydrate and protein metabolism,[13–21] genetic control of kernel metabolism,[2,22–29] regulation of kernel development,[30] and various specialty types of corn.[2, 31–33] High-quality photographs of the kernel phenotypes for many of the mutants can be found in the two books *The Mutants of Maize* and *Mutants of Maize*.[34,35]

II. HISTORICAL PERSPECTIVE

As with other major cereal crops, the origin of corn as a domesticated crop was prior to the written record. It is clear that corn had evolved all the morphologically essential features that distinguish it from its nearest relative, teosinte, by the time of Columbus.[36–39] By that time, the corn crop had spread throughout the New World from South America to as far north as the Canadian border of North America. In many of the religions of Native Americans, the corn god or goddess was the supreme deity and corn itself was a gift of the gods.[40] The Native Americans also had developed large numbers of varieties and specialty corns by this time.[38,39] In fact, all of the major types of corn — including dent, flint, flour, pop, and sweet corn — had been developed[39,40] and were used in early attempts by botanists to classify races and varieties of corn.[36,41] Similarly, the practice of isolation of varieties was sufficiently well developed to allow the maintenance of the genetic purity of the specialty types that are controlled by recessive genetic characters. The Native Americans had developed a number of specialty uses for the various types of corn as well. Different corns were used for the production of flour, beverages, soups, dried candies, and other purposes. For example, sweet corns were eaten green as a highly prized fresh product; immature kernels were parboiled and/or dried to produce a candy, *kancha*, and boiled and dried for eating during the winter months; mature kernels were ground to produce the confection *pinole* and as a fermentable source for the production of an alcoholic drink, *chicha*.[38,42,43] In fact, green corns were based on sugary and flour corns.[40]

III. THE GENETIC SYSTEM

Since the rediscovery of Mendelian principles of inheritance shortly after the turn of the century, corn has been the subject of intense genetic analysis. Collins and Kempton[44] described the *waxy1* gene and the recessive behavior of this gene. After this report many additional genes modifying kernel appearance and other characters were identified by genetic analysis.[45–48] The fruits of these efforts are the large numbers of genes identified and mapped to the ten chromosomes of corn. During this period, genes were identified based on the distinct morphological character (the phenotype) conditioned by the mutant genotype. The nomenclature for the genes was based on a description of this mutant character. In addition, a gene symbol of one or two letters was assigned to the gene. The rules of nomenclature for maize genes was standardized in 1974, and revised in 1995 and 1997 (see Neuffer et al.[35]).[49,50]

A general practice is to only list the mutant genes when describing a genotype. It is assumed that unless a gene is listed, the genotype at that locus is homozygous for the wild type allele. For example, when a *waxy1* (*wx1*) cultivar is referred to, it is assumed that the other genes influencing kernel carbohydrates are wild type. This will be the format used throughout this chapter. A second result of the intense genetic analysis of maize has been the identification of multiple alleles for various genes. These alleles are given a laboratory number or designation that is separated from the gene symbol or name by a dash.

More recently, genes have been identified based on their biochemical phenotype, i.e., the protein product which the gene encodes. When genes are identified and described on this biochemical basis, there is not necessarily a morphological characteristic of the kernel associated with allelic variation of the gene. As a result, these genes are named based on the protein product of the gene (i.e., *Zein protein, Zp1;* and *Sus1*, constituitively expressed sucrose synthase).

Based on the characterization of the effect of various genes on the properties of the kernel, the mutants can be divided into several groups. Gene systems which influence kernel development, kernel color/pigmentation (anthocyanins and carotenoids), kernel carbohydrates, proteins, and lipids will be discussed in this chapter. Because kernel carbohydrates and proteins are of major concern for their potential for the production of specialty corn, the major part of the chapter will be devoted to these components of the kernel and mutants affecting their quantity and quality.

A. Genes Affecting Starch and Protein Composition of the Kernel

1. Genic Variation

Many of the genes identified early in the genetic analysis of corn were genes affecting the appearance of the kernel. These mutations were easily identified, and the genetic segregation could be observed on a single F2 ear. The description of these genes beginning, with *waxy1,*[44] Was based primarily on the altered appearance of the mutant kernel when compared with the nonmutant or normal kernel phenotype (Tables 1.1 and 1.2). Essentially all of these mutants were recessive and required the homozygous genotype to be expressed. Occasionally, dosage effects were observed where the phe-

TABLE 1.1
Corn Genes Affecting Carbohydrate Composition of the Kernel

Gene[a]	Gene Symbol	Chromosome	Kernel Phenotype[b]
amylose extender1	*ae1*	5	tarnished, translucent, or opaque; sometimes semi-full
brittle1	*bt1*	5	shrunken, opaque to tarnished
brittle-2	*bt2*	4	shrunken, opaque to tarnished
dull1	*du1*	10	opaque to tarnished; S.C.[c] semi-collapsed translucent with some opaque sectors
miniature seed1	*mn1*	2	small, somewhat defective kernel, viable
shrunken1	*sh1*	9	collapsed, opaque
shrunken-2	*sh2*	3	shrunken, opaque to translucent
shrunken-4	*sh4*	5	shrunken, opaque
soft starch1	*h1*	—	opaque
sugary1	*su1*	4	wrinkled, glassy; S.C. not as extreme
sugary-2	*su2*	6	slightly tarnished to tarnished
waxy1	*wx1*	9	opaque

[a] All gene loci are named and symbolized using the revised rules for genetic nomenclature.[35]
[b] Adapted from Garwood and Creech.[56]
[c] S.C. Sweet corn background differs from dent background.

Source: From Hallauer, A.R., *Specialty Corns*, CRC Press, Boca Raton, FL, 1994. With permission.

TABLE 1.2
Corn Genes Affecting Protein Composition of the Kernel

Gene[a]	Gene Symbol	Chromosome	Mature Kernel Phenotype
floury1	*fl1*	2	opaque[a]
floury2	*fl2*	4	opaque[a]
floury3	*fl3*		opaque
opaque1	*o1*	4	opaque
opaque2	*o2*	7	opaque
opaque5	*o5*	7	opaque
opaque6	*o6*		opaque; lethal seedling
opaque7	*o7*	10	opaque
defective endosperm—B30	*De-B30*	7	opaque
mucronate1	*Mc1*	?	opaque

[a] These loci show dosage effects to varying degrees.

Source: From Hallauer, A.R., *Specialty Corns*, CRC Press, Boca Raton, FL, 1994. With permission.

notypes of the heterozygous kernels were intermediate to the phenotypes of kernels homozygous for either allele. Kernel phenotypes/gene names were described based on the overall appearance (i.e., *waxy1*, *dull1*, *shrunken1*), the light transmission properties of the kernels (i.e., *opaque1*), or the physical properties of the kernel (i.e., *brittle1* and *floury1*). In some instances, gene names were based on some desirable characteristic of the composition of the kernel, e.g., *sugary1* and *amylose extender1*. With further analysis, these genes have been shown to have major effects on the accumulation of the carbohydrate or protein fractions of the kernel. Those mutants affecting the carbohydrates of the kernel fall into two groups. The first group (Class One) includes the *shrunken*, *brittle*, and *miniature seed* mutants. The *shrunken* and *brittle* mutants have extreme phenotypes and greatly reduce the total dry weight of the kernel.[51,52] *Miniature seed* kernels have approximately one fifth of the dry weight of normal kernels.[53] The second group of mutants (Class Two) have a more varied array of phenotypes (*tarnished*, *opaque*, and *translucent*) and less effect on the total dry weight.[54–56] The genes influencing kernel protein fractions in general have opaque mature kernel phenotypes (Table 1.2).

With the identification of increasing numbers of new mutants, mutants were identified that had phenotypes similar to existing mutants but were, in fact, mutations of different genes. Standard allele tests (tests of genetic complementation often termed cis–trans tests) were required to ascertain whether a new mutation was due to either allelic variation or a new mutation.[57] In these tests, the new mutant is crossed to a known genetic stock of the previously identified mutant. If the F1 kernels had the mutant phenotype, allelism was confirmed. If the F1 kernels had a normal phenotype (complementation), allelism would be questioned and advanced generations (F2 and backcross, BC) would be analyzed. In those instances where the new mutant was found to be nonallelic to the original mutant, the new gene would be assigned the same gene name but be distinguished from the older gene by a number (i.e., *sh1*, *sh2*, and *sh4*). It is now recommended that the originally identified mutant carry the number one, e.g., *sh1*, *shrunken1*.[35,50] As this is a new convention, many researchers have not included the number "1" when describing the first mutant in a series. Therefore, unless designated otherwise, the reader should assume that the absence of a number with a gene name implies that the gene is the originally identified mutant (e.g., *sugary* = *sugary1*, *sh* = *sh1*, *brittle* = *brittle1*). In some instances, gene names and numbers were reported before allelism was tested and the gene was later shown to be allelic to another gene. In these instances, the originally reported name would be retained and the more recently reported gene name (and number) would be removed from use. For example, *o6* and *proline-1* were recently shown to be allelic.[58] In this case, the original name, *o6*, was retained. As a result, some gene names/numbers are not complete series (e.g., no *shrunken-3* or *opaque-3* gene exists). A recent report using Robertson's *mutator*

stocks to generate mutations demonstrates that even after 85 years of new study, new mutations are likely to continue to be identified and characterized.[59]

The phenotypes conditioned by different mutants are not always the same in different genetic backgrounds. This can often be observed when the mutants are backcrossed into different inbred backgrounds. For example, the phenotypes of *su1* kernels are highly variable.[56] The phenotype of *su1* kernels in sweet corn inbreds is often less extreme than in dent corn inbreds. In some instances, *su* kernels in dent lines can be almost as extremely wrinkled and collapsed as Class One mutants, *sh2* and *bt2*.[60] In many instances, these extreme kernels are difficult to germinate under field conditions.[61–63] In some backgrounds, other mutants (i.e., *ae1*, *su2* , *fl1*, *fl2*, and *du2*) give kernel phenotypes that are difficult to distinguish from the normal kernels on a segregating ear.[64] In these instances, the genotype of a line is best confirmed by allele testing with a line where the mutant produces a strong phenotype.

The variation with genetic background can be due to quantitative genes differing between lines that are accumulated during the breeding process or possibly to a fewer number of modifier genes, each of which might have only small effects on the expression of a major gene. This variation can be of significant importance in the utilization of the genes in specialty corn production. For example, the apparent amylose content of *ae1* lines was increased significantly by 20% (50% apparent amylose to 70%) by selection.[65,66] The differences in amylose content have been proposed to be due to one or few modifier genes selected by the breeder.[67] In sweet corn breeding, a specific modifier of the *su1* gene has been identified, named, and originally mapped to chromosome 4.[68–70] This modifier, *sugary enhancer1* (*se1*), is difficult to positively identify in all *su1* backgrounds, but usually *se1* has been associated with a lighter kernel color and a slower kernel drying rate on ears segregating for *se1*.[69,71] In addition, *su1* has been shown to affect sucrose and maltose concentrations in developing kernels. Recent Restriction Fragment Length Polymorphisms (RFLP) analysis has shown that a Quantitative Trait Loci (QTL) controlling these traits maps to the distal portion of the long arm of chromosome 2.[71] Numerous sweet corn cultivars have been developed using this modifier (Chapter 6). Modifier genes also have been selected in mutants affecting kernel proteins. The kernels of *o2* are of poor and unacceptable quality due to their soft, low-density kernels.[72–74] By selection, modifiers of *o2* which give hard kernel types have been developed.[75] Two modifier loci have been identified by genetic analysis.[76,77] These modifiers seem to specifically increase the synthesis of gamma-zein proteins in the kernel.[78–82] While these modifiers also increase gamma-zein synthesis in normal endosperm (*O2*), this increase does not modify the kernel phenotype.[83] One of these modifiers may be the (*o15*) locus.[84]

In addition to examining kernel phenotypes of single genes, many of the genes have been combined in sets of two or more genes. These multiple gene combinations show a number of genetic interactions (Table 1.3). These include epistasis, in which the kernel phenotype of the multiple gene combination is indistinguishable from the phenotype of one of the single genes. For example, *bt2* is epistatic to *su1* and kernels of the double mutant *bt2 su1* are shrunken. In the double mutant *su1 wx1*, *su1* is epistatic to *wx1* and the kernels are wrinkled and glassy. Other gene combinations have been described as showing complementary interactions, in which the phenotype in the multiple genotype is distinct from the phenotype of either single gene in the combination.[56] The double mutant *ae1 su1* is a good example of a complementary interaction. The *ae1 su1* kernels are translucent and glassy but not as wrinkled as *su1* kernels or as full as *ae1* kernels. As additional mutant genes are added to the genotype, the phenotype generally becomes more severe. As a result, many of the phenotypes of the multiple gene combinations are difficult to clearly differentiate. Earlier, Boyer and Shannon[2] divided the mutants into two classes. Class One mutants have the most extreme phenotypes and include the brittle and shrunken genes. These genes are epistatic to other genes or Class Two mutants in multiple genotypes. Within Class Two mutants, various forms of complementary or epistatic gene action are observed.[56] However, when multiple Class Two mutants are combined, the phenotype becomes more extreme as additional recessive alleles are added to the genotype. In many instances, the phenotypes of kernels with multiple Class Two mutants are as extreme as Class One phenotypes. It should be noted that epistasis only is being

TABLE 1.3
Examples of Gene Interaction in Double and Triple Mutant Gene Combinations for Genes Affecting Kernel Carbohydrates and Proteins

Genotype	Interaction	Mature Kernel Phenotype[a]
ae1 bt1	epistasis (*bt1*)[b]	shrunken, opaque to tarnished
bt2 su1	epistasis (*bt2*)	shrunken, translucent to tarnished
sh1 su1	complementary	extremely wrinkled, glassy with opaque sectors
o2 sh	epistasis (*sh1*)	collapsed, opaque
ae1 sh2 wx1	epistasis (*sh2*)	shrunken, opaque
su1 wx1	epistasis (*su1*)	wrinkled, glassy to opaque
ae1 su1	complementary	not as full as *ae*, translucent (tarnished in S.C.[c]) may have opaque caps
ae1 o2	epistasis (*o2*)	opaque, tarnished sectors in W23 × L317 background
fl1 sh2	epistasis (*sh2*)	shrunken, opaque
du1 fl1	epistasis (*fl1*)	opaque
ae1 su1 su2	complementary	partially wrinkled, translucent to tarnished
ae1 du1 wx1	complementary	shrunken, opaque to tarnished; S.C. semi-collapsed opaque

[a] Based on Garwood and Creech.[56]
[b] Gene expressed is given in parentheses.
[c] S.C. Sweet corn.

Source: From Hallauer, A.R., *Specialty Corns*, CRC Press, Boca Raton, FL, 1994. With permission.

considered for the phenotype of the mature kernel. Epistasis at the level of kernel composition is described in Sections IV A and D.

As the kernel phenotypes become more extreme, it becomes more difficult to reliably identify the kernel genotypes based on phenotype. This difficulty is further compounded by the frequent occurrence of dosage effects in multiple gene combinations.[85,86] In this regard, the triploid nature of the endosperm is important. The triploid condition derives from the fertilization of two 1n polar nuclei in the egg sac by one 1n sperm nucleus from the pollen. As a result, the endosperm can have four genotypes when two alleles are segregating at a locus, e.g., *Wx1 Wx1 Wx1*, *Wx1 Wx1 wx1*, *Wx1 wx1 wx1*, and *wx1 wx1 wx1*. When only the *wx1* gene is segregating, the first three genotypes produce kernels with the normal phenotype and the expected 3:1 genetic ratio (3 normal kernels to 1 *wx* kernel) is observed. However, if the kernel is homozygous for a second recessive allele (i.e., *ae1*), each of the four *wx1* genotypes sometimes produce "slightly different" phenotypes, and the different phenotypes become difficult to distinguish.[85] Similar results are observed when *ae1* is segregating in a homozygous *wx1* background. When both *wx1* and *ae1* are segregating on the same ear, many phenotypes are observed (16 genotypes are expected). These dosage effects, as well as the lack of distinct phenotypes in many multiple gene combinations, necessitate that the genotypes of these gene combinations be confirmed by tests of allelism. We recommend that the tests be performed for all genes thought to be present in the genotype.

By making appropriate choices in selecting for multiple genotypes, the researcher can often improve the chances of success for obtaining the desired genotype. This is frequently done by the selection of kernels with the phenotype of the least extreme single mutant from the segregating ear. For example, in the case of the joint segregation of *ae1* and *wx1*, only 1 of 16 of the kernels would be expected to be the double mutant *ae1 wx1*. Because errors in selection of *ae1 wx1* kernels would be expected due to the dosage effects discussed above, many of the selections will continue to segregate. By selecting *wx1* kernels from the segregating ear, an average of two out of three of these kernels would be expected to be heterozygous for *ae1* while homozygous for *wx1*. In the next generation, an ear of *wx1* kernels segregating for *ae1* would be produced. This ear would have one fourth of the kernels *ae1 wx1*. In addition, the *wx* background is clearly tested on the ear and the uncertainties that may arise from ear-to-ear or line-to-line variation can be reduced. Again, proof of the final genotype should be confirmed by appropriate allele tests.

Multiple combinations of genes influencing the protein fractions have also been developed. As with the single mutations, the kernel phenotypes are opaque. Various types of interactions are seen when the protein mutants are combined with the mutants affecting kernel carbohydrates (Table 1.3). The Class One mutants that affect kernel carbohydrates are epistatic to the protein mutants for kernel fullness. However, in some instances (i.e., *sh1 o2*) the double mutant is clearly distinguishable from either single mutant (Table 1.3). Phenotypes of double mutants of Class Two carbohydrate mutants and protein mutants are generally opaque or opaque with tarnished sectors (Table 1.3).

2. Allelic Variation

The genetic analysis of newly occurring mutations resulted in the identification of multiple alleles at many of the gene loci. Often, the phenotypes of these new alleles were indistinguishable from the phenotypes of existing alleles. In some cases, these alleles have been shown to be similar with regard to their effects on the chemical composition of the kernel as well.[87,88] Some allelic series, however, include novel alleles, which are now being exploited for gene cloning and may have potential for specialty corn. The most powerful alleles for gene cloning are those which result from the insertion of a transposable element in the coding regions of a gene or near a gene.[89–94] These alleles are often unstable and produce a variegated phenotype. In addition to being important to unraveling the events involved in transposition of the element, these alleles have provided the means to isolate genes without complete knowledge of the biochemical/metabolic function of the gene (see Section IV). Stocks of Robertson's *mutator* also have proven a rich source of new variation.

In other instances, alleles of a particular gene have been shown to have distinct kernel phenotypes and composition. Interestingly, these alleles have been relatively unused in specialty corn breeding. The *waxy1* gene locus was thoroughly investigated by Oliver Nelson and his associates. He identified and collected many alleles at this locus. In some cases, these alleles were functionally intermediate and had chemical phenotypes between *waxy1* and normal kernels. For example, kernels homozygous for *wx1-a* allele were shown to have 4 to 5% amylose as compared to 0 and 25% amylose in *wx1-c* (the standard allele) and normal kernels, respectively.[95] To our knowledge, no attempt has been made to use these alleles in specialty corn production. Hannah and Bassett[96] reported that an allele of *brittle1*, *bt1-A*, had unusually good germination for these Class One type mutants. At the same time, the sugar levels of this allele were as high as other supersweet corn types. These properties were suggested to be of potential use for the development of supersweet corn with improved field germination. Again, no commercial use of this allele has occurred at this time. The laboratory of Hannah identified a number of alleles that resulted from the mutation of *shrunken2* containing the transposable element *dissociation* (*Ds*).[97] When *Ds* is removed from the gene, novel alleles arise when three to six new base pairs remain in the gene. One of these alleles, *sh2*–Rev 6, produced seed with 11 to 18% greater weight. The observed phenotype was explained by modified allosteric properties from the enzyme ADP-glucose pyrophosphorylase, which is partially encoded by *sh2* (see Section IV). Similar results have been observed in potato transformed with allosteric-modified ADP-glucose pyrophosphorylase from *E. coli*.[98] The potential for *sh2*-Rev 6 to increase yield is being investigated at this time. As additional molecular analysis of different genes occur, it is likely that variations in "wild-type" and natural alleles will become increasingly obvious. A number of examples at the O2 locus have recently been reported.[99,100] However, the practical value of these alleles for specialty corns remains to be demonstrated.

The most dramatic variation of alleles has been observed at the *sugary1* locus.[60,101–104] Alleles of *su1* can range from glassy wrinkled to almost normal in phenotype (Table 1.4). To date, the alleles at the *sugary1* locus fall into three distinct classes. Twelve of these alleles collected at Pennsylvania State University by Roy Creech have phenotypes indistinguishable from the *su1*-Ref allele and have easily identifiable glassy wrinkled kernels. Three alleles, *su1*-am, *su1*-66, and *su1*-P, have full kernels that are indistinguishable from the normal or wild type kernels. These alleles

TABLE 1.4
Allelic Variation at the *sugary1* Locus of Corn

Allele	Name	Mature Kernel Phenotype	Composition[a] (mg/g dry weight)		
			Sucrose	Starch	Phytoglycogen
Su1-Ref	*reference*	wrinkled, glassy	245	77	130
Su1-st	*starchy*	wrinkled, glassy crown full	124	191	122
su1-Bn2	*brawn2*	wrinkled, glassy less extreme then *su-Ref*	177	241	55
su1-am	*amylaceous*	full — near normal	78	356	4

[a] 20 days after pollination; adapted from Garwood and Vanderslice.[101]

Source: From Hallauer, A.R., *Specialty Corns*, CRC Press, Boca Raton, FL, 1994. With permission.

are best observed in double mutant combinations with *su2* and *du1*. In these combinations, the kernel phenotypes included glassy wrinkled (*su1*-66 *su2*) and extremely shrunken with opaque sectors similar to Class One mutants (*du1 su1*-P) and collapsed glassy (*su1*-P *su2* and *su1*-am *su2*). Two alleles, *su1*-st and *su1*-Bn2*,* are intermediate to the full and standard sugary phenotypes. The phenotypes of these kernels are more variable and generally include a partially wrinkled crown of the kernel. The chemical composition of these kernels varies with the physical phenotype as well (Table 1.4). One study evaluated the potential of the *su1*-Bn2 allele for improved silage corn.[105] It was reasoned that the intermediate phenotype of this mutant would provide enhanced nutritional value to the silage (primarily available carbohydrate). A more recent study found no improvement of *su1*-Bn2 over dent corn as a part of the alfalfa silage mix for dairy cows. In fact, this study reported adverse effects on ruminal forage digestion.[106]

B. Genes Affecting Kernel Color

The appearance of mature kernels can range from white to almost black. Two pigments are primarily responsible for determining the color of the corn kernel. The carotenoids are primarily associated with the yellow color of the kernel. Two classes of carotenoids are the carotenes and xanthophylls. Carotenoids are important as sources of vitamin A in the diet and xanthophylls as feed sources for the yellow color of poultry skins and egg yolks.[107] The major carotene is B-carotene.[108] The most abundant xanthophylls of the kernel are lutein and zeaxanthin.[108,109] The second class of kernel pigments is the anthocyanins, which are red to blue in color. Anthocyanins are flavonoid compounds of which cyanidin, pelargonidin, and peonidin are found in the kernel. Additional variation of the anthocyanins is derived from different types, combinations, and degree of hydroxylation, glucosylation, methylation, and acylation.[110] Reviews and extensive treatments of the genetic control and biochemistry of these pigments in corn have been recently published.[12,110,111] Therefore, only the major genetic variation will be considered here.

Many genetic loci determine the color of corn kernels. Based on the results of numerous researchers, the genes modifying anthocyanin content of the kernels have been ordered in the pathway of synthesis.[110–112] These gene orders are based on both biochemical and genetic studies. Based on the placement of a mutant, color and pigment content can be explained. Several loci (*C1*, *colored aluerone1*; *c2*, *colorless 2*; *a1*, *anthocyaninless1*; *a2*; and *R1*, *colored1*) condition the absence of color if a homozygous recessive genotype is present for any of these loci. Some of these loci appear to be regulatory, based on numerous criteria, whereas other loci have been identified as structural genes for specific enzymes in the biosynthetic pathway.[111] Many alleles have been identified for these loci, and these alleles not only modify anthocyanin accumulation in the kernel but other plant tissues as well.[12,30] The effects of the loci *bz1*, *bronze1,* and *Bz2* are seen when the dominant alleles of the loci above are present. The homozygous recessive condition at *bz1* or *bz2* results in brown kernels, while kernels of the double mutant are colorless.[12] Quantitative variation in color has also been described at the genetic level. The recessive allele of *In1*, *intensifier1,*

increases pigmentation when homozygous.[30] The dominant *pr1* (*red aluerone1*) conditions a high ratio of cyanidin glucoside to pelargonidin glucoside.[30,113]

In addition to accumulating in the kernel, carotenoid pigments are also important components of photosynthetic tissues. Coe and Neuffer[12] classified 117 loci affecting photosynthetic pigments including the carotenoids. In this classification, mutants are classified on endosperm color (yellow vs. pale or white), embryo dormancy (dormant vs. viviparous), seedling color (green, yellow–green, yellow, white, etc.), and plant color (green, yellow–green, etc.). Four loci (*Y1*, *yellow1*; *y8*; *c1*, *white cap1*; and *Bn1*, *brown aluerone1)* have mutant phenotypes that are white or pale kernels, dormant seeds, and green seedlings and plants. When the recessive alleles are homozygous for *y1*, *y8*, or *bn1,* the kernels are white or pale yellow.[114] In contrast, *Wc1* is a dominant mutation and gives kernels with white crowns and pale yellow endosperm when the *Y1* allele is also present.

Other mutants affecting kernel carotenoids also have white or pale yellow endosperms. In addition, these mutants affect seed dormancy, and seedling and plant color.[12] Many of these mutants are viviparous (precocious germination), including *y7*, *y9*, *vp2* (*viviparous-2*), *vp5*, and *w3* (*white-3*).[12,30] One *viviparous* mutant, *vp,* has a similar pleiotropic effect on anthocyanin accumulation in the kernel.[30] The pleiotropic effects can be explained in part by a common early pathway for the carotenoids and abscisic acid.[115,116] Finally, mutants including *y10*, *cl1* (*chlorophyll*), and the *lemon white* mutants (*lw1*, *lw2*, *lw3*, and *lw4*) have albino or pale yellow seedlings that die after germination.[12]

C. Genes Affecting Kernel Development

In addition to the mutants described that have abnormal dormancy, mutants frequently have been identified which affect early kernel development.[47,53,117–121] These mutants can affect embryo and endosperm development (*defective kernel*, *dek*, mutants), endosperm development, *(defective endosperm*, *de*, mutants), and embryo development (*embryo specific*, *emb*, mutants). Many of the *dek* mutants alter auxin and cytokinin levels, thus changing endosperm development.[122] Some mutants have been given more descriptive names based on phenotypes.[123] Because most of these genes are lethal and the mutant must be maintained as a heterozygote, many of the mutants identified in earlier studies have not been maintained. Recently, Nueffer and Sheridan[124–129] have assembled a large number of samples of embryo development in different mutants after mutagenesis. These mutants affect embryo development at many different times and in different ways. Some of these mutants can be "germinated" by placement of immature embryos in culture. Those mutants showing some development under these conditions were termed "nutritional mutants," while mutants failing to show some development under these conditions were termed "developmental mutants."[101] From this work, 51 embryo-specific mutations have been identified.[130]

The mutants affecting endosperm development have also been shown to differentially affect development in other tissues. The *miniature seed1* (*mn1*) mutant is characterized by premature breakdown of the placental–chalazal cells at the base of the kernel around 14 days after pollination.[53] This breaks the continuous bridge between the maternal and endosperm cells. This developmental breakdown, in turn, may be the result of osmotic damage from an invertase deficiency in maternal and endosperm cells.[131] Other mutants delay the initiation of dry matter accumulation or reduce dry matter accumulation at various stages of kernel development.[132,133] These variable mutant effects help to describe the genetic control of endosperm development.

IV. BIOCHEMICAL ANALYSIS

A. Kernel Carbohydrates

The mature corn kernel is composed of over 70% starch. By far most (80% to 90%) of this starch is found in the endosperm, which comprises about 80% of the total kernel dry weight. Starch is a homopolymer of glucose. The molecular structure involves only two linkages of the glucose

molecules: α-1,4 and α-1,6. A majority of the linkages are α-1,4; these linkages make up a linear arrangement of the molecules. The small amount of α-1,6 linkages provide a branching pattern to the molecule. Two distinct types of polymers are found in most starches. Amylose is essentially a linear molecule of α-1,4 linked glucose. Both linear and slightly branched amylose molecules are found in starch.[134–136] In the branched amylose molecules, the long stretches of linearly linked glucose are interrupted with a branch (α-1,6 linkage) about every 200 glucose units. In general, amylose molecules are highly dispersed in molecular weight. They range in size from 100 to 1000 glucose subunits, with the larger molecules usually containing a few α-1,6 branch points.[134] Amylopectin molecules are much larger than amylose and contain up to 200,000 glucose subunits. About 4% to 5% of the total glucose units of amylopectin are linked α-1,6. The linear regions of the molecule are short chains of various sizes with two populations of chain sizes of 10 to 20 glucose units and 30 to 50 glucose units.[137] This arrangement of the chains gives amylopectin a cluster-like structure.[138] The reader is referred to Chapter 2 for further details of the structure and functional features of starch.

Although starch, on initial examination, is a fairly simple polymer to understand, the synthesis of starch is still a subject of intense study. Several factors contribute to the complexity of the process of starch synthesis. These include the synthesis of starch as part of a megamolecular assembly, the starch granule. The starch granule is cold-water insoluble, while the majority of the enzymes involved in starch synthesis are soluble. The granule is composed of large numbers of amylose and amylopectin molecules (as many as 10^9 and 10^7, respectively). Furthermore, these molecules are regularly arranged, giving the granule stability and crystalline properties. Starch is not synthesized in the cytosol of the cell, but within the subcellular organelles, the amyloplasts. These double-membrane-bound organelles compartmentalize the process of synthesis, allowing only certain precursors for starch entry into the amyloplasts. As a result, the complete process of starch synthesis and accumulation in the corn kernel involves not only the direct synthesis of starch from glucose, but also the integrated metabolism of the sugar intermediates.

The complex integrated pathway of starch synthesis accounts for the large number of genetic loci affecting kernel carbohydrate composition. As these loci are expressed in a coordinated way during development, it is not surprising that complete complex interactions between mutants may occur.[139–141] Furthermore, it is the complete understanding of the nature of starch synthesis and all the associated genetic loci mutants that will allow the future manipulation of the process for the development of specialty corns based on starch types. Many reviews have proposed pathways and described the enzymes of starch biosynthesis.[14,16,18,23,27–29] We previously proposed looking at the pathway as containing two types of reactions (Figure 1.1).[2] In this approach, early reactions in the pathway are involved in the interconversion of sugar intermediates and the synthesis of the immediate substrate for starch synthesis, ADP-Glc. These reactions are often controlled by the Class One mutants discussed above which have extreme kernel phenotypes. Many of the reactions as drawn in the pathway are, in fact, catalyzed by different enzymes found in the cytosol or amyloplast.[142] The second part of the pathway includes the reactions directly involved in the synthesis of the starch molecules from ADP-Glc. Two basic reactions are involved. The starch synthase enzymes transfer a glucose moiety from ADP-Glc to the nonreducing end of an α-1,4 chain of the starch molecule, thus increasing the length of a linear portion of the molecule by one glucose unit.[143,144] The starch branching enzymes form the α-1,6 branch points of starch by breaking an internal α-1,4 linkage from a linear chain. The released chain (six or seven glucose units long) is then transferred to another chain by the formation of the α-1,6 linkage.[145,146] These enzymes have been shown to be the gene product of the Class Two mutants, such as *ae1* and *wx1*. The less extreme phenotypes of these mutants can be explained by the observation that the starch synthases and branching enzymes exist as multiple forms. Therefore, the elimination of an enzyme activity by a given mutant only partially reduces the total activity.

The epistatic masking of the phenotypes of Class Two mutants by Class One mutants is explained by the early position in the pathway of the enzymes encoded by Class One mutants. The

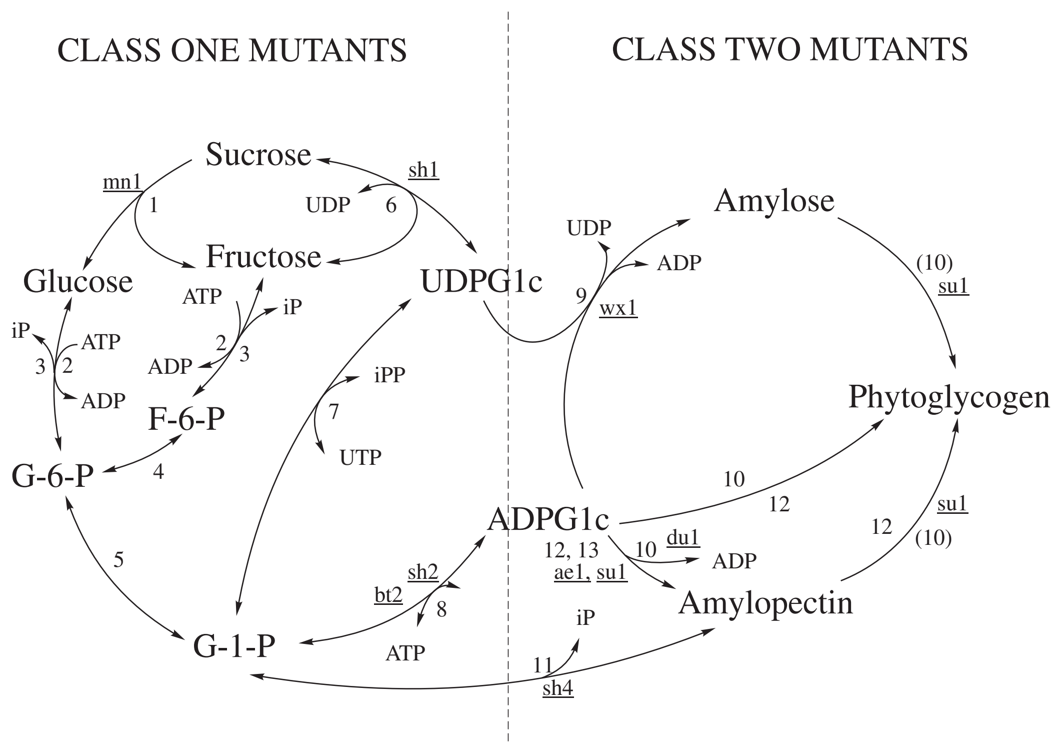


FIGURE 1.1 Hypothetical scheme for the formation of starch and phytoglycogen from sucrose in corn kernels. Location of various mutants affecting carbohydrate accumulation in the pathway is shown. These mutants may be structural genes for the enzymes indicated or have secondary effects on the enzyme activities. Enzyme reactions are indicated by number as follows: (1) invertase (EC 3.2.1.26), (2) hexokinase (EC 2.7.1.1.), (3) hexose-6-phosphatase (EC 3.1.3.9), (4) glucose phosphate isomerase (EC 5.3.1.9), (5) phosphoglucomutase (EC 2.7.5.1), (6) sucrose synthase (EC 2.4.1.13), (7) UDP-glucose pyrophosphorylase (EC 2.7.7.9), (8) ADP-glucose pyrophosphorylase (EC 2.7.7.27), (9) starch granule bound starch synthase (EC 2.4.1.21), (10) soluble starch synthase (EC 2.4.1.21), (11) starch phosphorylase (EC 2.4.1.1), (12) starch branching enzyme (Q-enzyme) (EC 2.4.1.18), (13) starch debranching enzyme (isoamylase-like) (EC 3.2.1.68). Modified from Boyer and Shannon.[2]

specific lesion of *sh1* kernels is sucrose synthase (reaction 6). The enzymatic lesion of *sh2* and *bt2* is ADP-Glc pyrophosphorylase (reaction 8). As these reactions are in the earlier part of the pathway, the reduction of starch synthesis is seen regardless of the genotype at the Class Two loci. The *sh4* gene was initially shown to have lowered activities of starch phosphorylases, which at one time was believed to be a starch polymerizing as well as a degradative enzyme.[147] However, the lesion of *sh4* has been located in the synthesis of pyridoxal phosphate.[148] Pyridoxal phosphate is a cofactor for many enzyme reactions, including phosphorylase; therefore, *sh4* kernels show multiple altered enzyme activities.

Three additional points should be made and discussed from examination of the pathway presented in Figure 1.1. First, despite the extensive genetic analysis of corn during the past 90 years, mutants for many of the enzymes in the pathway have not been found by the isolation of mutants based on kernel phenotypes. Two possible explanations for this exist. First, the actual pathway may not require the enzymes as developed in this pathway. Some support for this view has been presented based on the studies of the enzyme phosphoglucomutase (reaction 5, Figure 1.1). This enzyme exists in two forms in the endosperm.[142] One of the forms is found in the amyloplasts, while the other form is in the cytoplasm of the endosperm cell. Many standard inbred lines have been found to lack measurable activity of the cytosolic form of this enzyme.[149] As the kernels of

these inbreds are normal in appearance, the logical conclusion of these observations is that the cytosolic form of this enzyme is not necessary for the normal development of the kernel. A second possible explanation for the failure to identify mutants for many enzymes may be that the deficiency of these enzymes produces such a severe disruption of kernel development and starch synthesis that they prove lethal. Possibly some of these mutants may be identified in the *dek* or *de* mutants discussed in Section III C. *Miniature seed* is an example of a mutant with both developmental (placental–chalazal tissue breakdown) and biochemical (invertase, reaction 1, Figure 1.1) lesions.[131]

A second point is that Figure 1.1 also shows that more than one gene may be found to be associated with a particular reaction. Although many different suggestions have been made to account for these observations, most of the mutants can be explained by more than one structural gene for the enzymatic reaction. In the case of the enzyme ADPG pyrophosporylase, *bt2* and *sh2* kernels both contain about 10% of the activity found in normal kernels.[150–154] Both of these genes encode a subunit for the functional enzyme which is thought to exist as a tetramer of the two large subunits (*bt2*) and two small subunits (*sh2*).[155–157] The loss of a functional allele for either subunit reduces the observed activity. In the case of sucrose synthase, the *sh1* mutant also reduces the enzyme activity to about 10% of the activity in normal kernels.[158] In this case, a second enzyme is the product of a second gene, *Sus1*. The second gene was identified based on biochemical and genetic analysis. No mutant phenotype is known for this locus.[159,160]

The starch synthases and branching enzymes also are found to exist as multiple enzymes and, in some cases, the same form of these enzymes is found as both on soluble fractions and bound to the granule.[161] In addition, each of the multiple forms of starch synthesis and starch branching enzymes have slightly different enzyme properties and thus are likely to play different roles in starch biosynthesis.[162,163] The starch synthases are both soluble enzymes and starch-granule-bound enzymes.[143,144,164–166] The *wx1* mutant reduces the activity of the major starch-granule-bound enzyme.[143] A minor starch-granule-bound starch synthase and the soluble starch synthases are not affected.[144,167,168] Similarly, the starch branching enzymes have been shown to exist as two or three fractions.[165,166,169] It is clear that two enzymes exist: branching enzyme I and branching enzyme II. Branching enzyme II also has been further divided into two fractions, branching enzyme IIa and IIb.[165,166] These fractions have very similar properties including similar chromatographic affinities, amino acid composition, molecular weight, and peptide maps.[165,166,170,171] The *ae1* gene, however, only influences the activity of one of these fractions (branching enzyme IIb). The other branching enzyme activities are not influenced in *ae1* kernels. [165,166] The effects of dosage of the recessive *ae1* allele on branching enzyme IIb activity are consistent with the function of *ae1* as a structural gene of this enzyme.[172] Therefore, branching enzymes IIa and IIb are encoded by two very similar genes, only one of which is *ae1*.[173]

A third point from Figure 1.1 is that not all mutants have been clearly identified with a particular lesion at the biochemical level in spite of intensive efforts in many laboratories. In an earlier edition of this book, the biochemical explanations for *bt2*, *du2*, *su1*, and *su2* were still being intensely researched and their roles in starch synthesis were being debated. The *sugary1* locus of corn is probably the most extensively studied of any mutants due to its traditional importance in sweet corn cultivars. In the past 5 years, significant progress has been made toward understanding how *su1*, *du1*, and *bt1* affect starch synthesis. Thus the biochemical lesion for only *su2* remains to be explained. Starch from *su2* kernels has increased amylose content and unusual thermal properties.[174] As seen in the following discussion of *su1*, *du1*, and *bt1*, understanding the role of the *su2* levels may provide some surprises and changes in the current views of starch biosynthesis.

The major modification of the carbohydrate composition of *su1* kernels is the large quantities of phytoglycogen that are accumulated.[175–180] This phytoglycogen is similar to animal glycogens and is water-soluble. Over the years, several reports on a "phytoglycogen branching enzyme" in *su1* kernels have appeared.[181–186] These reports have appeared as recently as 1987.[185] However, in our studies we have found this "phytoglycogen branching enzyme" in all genotypes whether phytoglycogen accumulates or not.[165,166] More recently, Pan and Nelson[187] reported that *su1* kernels

contain less starch debranching enzyme than corresponding normal kernels. This observation supported the earlier suggestion of S. Erlander[188] that amylopectin was formed by the action of debranching enzyme on phytoglycogen. More careful characterization of the debranching enzymes in developing endosperm demonstrated that both pullulanase and isoamylase activities are present.[189] Cloning and careful characterization of the enzyme product of *su1* has clearly demonstrated that the *su1* locus encodes debranching enzymes with isoamylase-type activity.[190–193] The pullulanase activity is encoded by a separate gene, *zpu1*. While the direct activity of a debranching enzyme on phytoglycogen is not sufficient to produce amylopectin as originally suggested by Erlander,[188] the action of the *su1* debranching enzyme in combination with starch synthases and branching enzymes make up an enzyme complex responsible for amylopectin synthesis (see review[195]).

The *du1* mutant has been shown to decrease the activities of both soluble starch synthase and branching enzyme IIa activity.[165] In these early studies, it was impossible to establish either of these enzymes as the gene product of this locus. The great interest in starches from various combinations of *du1* and other kernel mutants[5, 8, 9] justify future biochemical studies of this locus. The amylopectin fraction of starches from *du1* endosperm have been shown to have shorter chain lengths than amylopectins from normal or *wx1* endosperm in numerous studies.[196,197] These observations are consistent with reduced starch synthesis activity. More recently, the cloning of the *du1* gene has already demonstrated that the gene encodes the major activity in one of the two soluble starch synthase fractions in developing corn endosperm.[197–199]

Characterization of the *bt1* locus has presented challenges and unexpected findings. Starch accumulation in kernels of this Class One mutant only reaches 25% of the starch of normal kernels.[200] An early report showed that *bt1* kernels were reduced in the activity of an enzyme that is capable of the formation of a short oligosaccharide from glucose-1-P.[201] This enzyme has been suggested as a key reaction in the formation of the initial oligosaccharides for the initiation (priming) of starch synthesis and a mutant in this enzyme would be expected to be a Class One mutant.[202] However, other priming reactions of starch synthesis have been proposed. For example, the primer for glycogen synthesis, glycogenin, has been identified.[203] Glycogenin is a self-catalytic protein that provides a protein primer for glycogen synthesis. A similar protein has been reported from phytoglycogen from sweet corn.[204] The role of this protein (or related proteins) in starch biosynthesis remains to be elucidated. These reactions do not, however, appear to be the function of the *bt1* gene product. Cloning of the gene showed partial homology with genes encoding proteins which transport adenylate across plastid membranes.[94] Other studies had demonstrated an adenylate translocator in maize amyloplast membranes.[205] Subsequently, it was shown that membranes from amyloplasts from *bt1* endosperm lacked three major related proteins[206,207] and that the *bt1* protein was target to the inner membrane of chloroplast.[208] As the major substrate for starch synthesis, ADP-glucose was thought to be synthesized in the amyloplast, an obvious explanation for the extreme phenotype of *bt1* difficult to account for initially. However, at the same time, other researchers found that ADP-glucose pyrophosphorylase subunits encoded by *sh2* and *bt2* were not modified after translation as would be expected of proteins transported into the amyloplast.[209] Thus the activity of this enzyme was located both in the cytosol and amyloplast.[210] Examination of sugar intermediates in *bt1* kernels revealed that these kernels contained 10 times the amount of ADP-glucose as wild-type kernels.[211] Thus the *bt1* kernels have reduced starch synthesis due to the failure of ADP-glucose synthesized in the cytosol to be transported into the amyloplast. These unexpected findings demonstrate the continued need for research on starch synthesis and critical examination of proposed pathways.

The pathway presented in Figure 1.1 is also useful in providing possible explanations of the changes in the quantity and type of carbohydrate accumulating in kernels of different genotypes. It is these changes which provide the basis for the use of various mutants for the production of specialty corn starches. An extensive review of the effects of various mutants and mutant combinations on kernel dry weight, starch granule size, and glucan composition (amylose, amylopectin,

and phytoglycogen) is available.[23] Therefore, we will only consider some of the key interactions and related points. The most consistent effect of various genotypes on kernel dry weight and starch granule size is a reduction of these in the mutant kernels compared with normal kernels. Notable exceptions are *wx1* kernels and starch granules, which have similar dry weights and size as normal kernels and starch granules. In this regard, it is important to consider that the amylose and amylopectin fractions of the starch are synthesized in separate processes. As *wx1* blocks the less efficient pathway, amylose synthesis, there seems to be sufficient potential of the amylopectin synthetic pathway to compensate for the lost synthesis due to *wx1*. Therefore, amylopectin synthesis in *wx1* kernels is actually increased.

The separation of amylose and amylopectin synthesis also explains the epistatic action of *wx1* in combinations with other mutants at the level of starch composition. All double and triple mutant combinations including *wx1* contain no amylose.[54,212,213] Early reports on the double mutant *ae1 wx1* suggested that this starch did, indeed, contain amylose.[54,212,214,215] However, these results were based on the measurement of amylose content using the blue value or a procedure based on iodine-binding of the starch. Subsequent analysis revealed that *ae1 wx1* starch was, in fact, composed solely of amylopectin, but this amylopectin had longer chain lengths than normal or *wx1* amylopectin.[216–219] This structure of the amylopectin results in a higher iodine binding, thus an overestimate of amylose based on this property of the starch. The structure of amylopectin in starch from *ae1* kernels is similar in structure to the structure of amylopectin from *ae1 wx1* kernels. The *ae1* kernels also contain the more obvious increased level of amylose. In *ae1* kernels where the efficiency of the amylopectin pathway is reduced, increased synthesis of amylose, therefore, occurs in a separate pathway. However, unlike the case in *wx1* kernels, this increased synthesis of amylose in *ae1* kernels is not sufficient to compensate for the lost amylopectin synthesis, and starch levels and kernel dry weight are decreased.

The relationship of amylose and amylopectin synthesis and the synthesis of phytoglycogen need to be considered as well. Neither the simple concept of amylopectin being the precursor to phytoglycogen nor phytoglycogen being the precursor to amylopectin seem to be valid. Studies of the development of *su1* kernels have shown that some phytoglycogen seems to be derived from the mobilization of starch granules (amylose and amylopectin).[220] However, additional synthesis and polymerization of phytoglycogen also is likely to occur. Detailed studies of the glucans from *su1* kernels have revealed a range of truly soluble molecules and particulates which could be removed by increasing speeds of centrifugation. These particulate fractions contained less phytoglycogen and more amylose and amylopectin as the size of the particulates increased.[175,178] Hence, these particulates likely represent various fractions of starch granules being mobilized to phytoglycogen. In the double mutant *su1 wx1,* phytoglycogen synthesis is slightly enhanced compared with *su1* kernels.[221] In contrast, *ae1* is epistatic to *su1* for phytoglycogen, and synthesis in the kernels of the double mutant *ae1 su1* is greatly reduced.[222] These interactions can be explained by the relative changes in the multiple activities of branching and debranching enzymes. In addition, the starch granules in the different genotypes are very different.[220,223] In particular, high amylose granules have been shown to be relatively resistant to amylase action in comparison with normal and *waxy1* starch granules.[224] This relative inert nature of the starch granules from *ae1* genotypes may be interfering with the mechanism leading to mobilization of the starch granule during phytoglycogen formation.

Although extensive studies on the properties of starches (and sugars) from kernels of various genotypes have been reported,[54,55,212,215,223–229] the reader should be aware that variation between genetic backgrounds has also been reported.[218,230–233] Most reports agree on the major properties of starches from a given genotype. However, comparison of various reports often show a range in the values of the different properties of the starch studied. In some instances, this range of variation may be attributed to different methodologies employed by the researchers. In recent studies on the various features of the starch, starch granule size and morphology, amylose content, amylopectin chain-length, and amylopectin molecular weight have been shown to vary as a result of the genotype

and the interaction of genotype and inbred line.[219,230–233] Furthermore, these properties could be correlated with the physical-functional behavior of the starches, including the temperature and energy required to swell the starch in aqueous systems.[230–233] Like the variation in mature kernel phenotypes for different genotypes in different backgrounds (Section III A), this variation in starch properties can be explained by either quantitative variation or modifier genes. The role of selection and modifier genes in amylose content of *ae1* kernels has already been discussed.[65–67] Another example of a modifier is the *se1* gene, which is a modifier of *su1*. The effects of *se1* are seen in the sugar content of immature *se1 su1* kernels, which are to two to three times the sugar levels of immature *su* kernels.[234–236] Certainly, the variation due to quantitative loci and modifier genes will be difficult to fix in specialty corns. However, with appropriate selection this variation should allow the plant breeder to fine-tune the properties of future cultivars.

B. Kernel Proteins

The proteins of the kernel are traditionally classified based on their solubility in different solvents as initially described by Osborne[237] and later modified by Landry and Moureaux.[238] Different researchers have applied varying conditions for the solubilization of proteins. As a result, differences in composition and complexity of the various fractions are in the literature.[15,25,26,239–244] The albumins are soluble in water and comprise about 7% of the whole kernel nitrogen. Globulins are soluble in salt solutions and comprise about 5% of the kernel nitrogen. The prolamins are soluble in alcohol with or without added reducing agents and comprise about 52% of the kernel nitrogen. Finally, the glutelins are soluble in dilute alkali and comprise about 25% of kernel nitrogen.

The major protein class, the prolamins, are collectively referred to as the zeins. These proteins contain large amounts of the amino acids glutamine, proline, leucine, and alanine, and are of relatively poor nutritional quality.[244–248] The zein proteins are typically separated after selective solubilization by polyacrylamide gel electrophoresis. This analysis reveals molecular weight heterogeneity with major components of MWs of 10, 14, 16, 19, 22, and 27 kD. Further heterogeneity is revealed by isoelectric focusing and gel electrophoresis.[249–253] One classification of zeins is primarily by molecular weight.[253] In this system, zeins are classified as A-, B-, C-, D- and E-zeins (21–26 kD, 18–24 kD, 13–18 kD, 9–11 kD, and 28 kD, respectively). More recently, the zeins have been classified based on the relatedness of their primary gene and amino acid sequences.[78,239,254–256] These studies classify the zeins as α-(22 and 19 kD), β-(14 kD), γ-(27 and 16 kD), and δ-(10 kD). The α-zeins are by far the most abundant fraction and comprise up to 80% of the total zeins. Zeins are synthesized by membrane-bound polyribosomes and transported to the lumen of the endoplasmic reticulum.[257,258] In the lumen, the zeins are assembled into insoluble protein bodies. Variation in the relative composition of the protein bodies in the aluerone and endosperm has been demonstrated.[259] The small protein bodies in the subaluerone contain β- and γ-zein and the larger protein bodies of the endosperm contain α- , β-, and γ-zeins.

The genes encoding the storage proteins are present as multiple copies; early estimates for the number of genes encoding the zein proteins ranged up to 100 genes.[260–268] It is not known whether all the genes are active in the synthesis of the zein protein, and different genes may be expressed at different levels.[269] The genes are distributed on several chromosomes in the corn genome. To date, 20 genes have been mapped to both arms of chromosome 4 and the short arm and long arm of chromosomes 7 and 10, respectively.[270–272] With further analysis, the relationship of gene families has become clearer. The α-zeins are encoded by a large gene family. In contrast, the β-, δ-, and γ-zeins are encoded by one or two genes.

The mutants affecting proteins of the kernel have been shown to affect zein levels to varying degrees.[88,241,273–280] The reduction in zeins in the mutant kernels is 20 to 50% of the level in normal kernels. Synthesis of all zeins are reduced in *fl2*, *Mc,* and *o6* kernels. In contrast, *o2* kernels and *De*-B30* kernels have more pronounced reductions of the 22 kD α-zeins, and *o7* kernels are greatly reduced in the 19 to 20 kD α-zeins. Although the various zein components are reduced in the

mutant kernels, it is still possible to identify all the different subunits (families) of zein. As a multigene family, mutation of a single α-zein gene would be expected to have little overall affect on the phenotype. Therefore, unlike the mutants affecting carbohydrate metabolism which usually encode enzymes of the biosynthetic pathway, the mutants influencing kernel proteins that have been identified based on kernel phenotypes are not structural genes for the storage proteins. These genes are regulatory, and the mutants affect the expression of complete sets of related genes encoding a particular class(es) of zein proteins.

Studies of the mutants have established that the amounts of zein mRNAs are decreased in mutant kernels.[275,281] Likewise, transcription of zein proteins is reduced in the mutants.[70] Various types of interaction of the mutants have been observed, and mutant effects are correlated to a "regulatory protein" called b-32.[280] More recently, the mechanism of the *o2* mutant has been described at a molecular level. The *Opaque2* protein is localized in the cell nucleus.[282] The *o2* locus encodes a DNA-binding protein with a leucine-binding motif.[283] This protein can bind the 5′ flanking sequences of the genes encoding the 22 kD α-zeins and acts as a transcriptional activator.[284] As this binding is thought to be part of the normal transcription process involving the *o2* encoded protein and other transcription factors, a mutant would be expected to reduce but not completely eliminate transcription of multiple α-zein genes.

The *fl2* mutation is associated with reduced synthesis of several classes of zeins.[275] This observation has resulted in speculation that the *fl2* mutation, like *o2* was involved in transcriptional regulation. However, *fl2* has several additional effects, including reduced membrane-bound polysomes,[275] the appearance of a novel 24 kD zein protein,[208] irregular-shaped protein bodies,[285] and elevated levels of a 70 kD cytoplasmic reticulum protein with homology to the chaperonin BiP.[286] Recently, the production of the novel 24 kD zein has been shown to be the result of a defective signal peptide in a 22 kD zein transcript.[287,288] This novel 24 kD protein would explain the altered protein bodies, while the alteration in the normal secretory process could result in the other associated changes in membrane polysomes and housekeeping proteins.[288] Biochemical explanations for other protein mutants remain to be elucidated.

C. Kernel Lipids

The amount and composition of lipids in corn kernels is also under genetic control. Corn kernels contain approximately 4.4% oil. In contrast, the Illinois High Oil line contained 20.4% oil after 85 generations of mass selection.[108] The increase in oil from 4.7% in the original population has been continuous over the course of selection and clearly demonstrates the potential genetic variability in corn for oil quantity. More recent studies have utilized NMR for rapid screening of kernels for oil content. Using this method, oil content was increased from 4.0% to 9.1% in seven generations.[289] Triacylglycerides are the major component of commercial corn oil, although a wide variety of other lipids are found in the kernel.[108] The triacylglycerides contain a mixture of saturated and unsaturated fatty acids. Corn oil is generally accepted as high quality as determined by a high linolenic acid (50%) and low linolenic acid (1%) content. Other fatty acids including oleic (40%), palmitic (12%), and stearic (2%) acid also are found in corn oil.

A number of different approaches have been used to demonstrate genetic variation for the fatty acid composition of the kernel. A survey of a wide range of germplasm sources showed that different lines ranged in composition from 14 to 64% for oleic acid and 19 to 71% for linoleic acid,[290] while exotic breeding materials were shown to have wide variation in fatty acid composition.[291] Analysis of U.S. commercial corn oil showed that the linoleic acid content increased from 55.8% to 62.8% in a 12-year period.[292] Most of this variation was attributed to changes in parental inbreds during the same time, although no direct selection for oil composition is practiced in most breeding programs. A single-gene *linoleic acid1* with a recessive allele, *ln1*, which conditions high linoleic acid levels, was identified in genetic studies involving Illinois High Oil corn.[293] Single-gene inheritance has also been identified in other reports.[294–296] Other studies using monosomic lines

have identified genes controlling oleic and linoleic acid composition on chromosomes 1, 2, 4, and 5.[297–299] High stearic acid and high oleic acid contents were reported to be under the control of one major gene.[300,301] In essentially all studies, researchers suggested that major gene effects were being modulated by modifier genes for oil composition. Although it seems that sources of major genes for composition of corn oil can be utilized, other studies indicate that the inheritance of oleic, linoleic, palmitic, and stearic acid content when considered together is complex and under multigenic control.[302–305] Additional studies are needed to more precisely identify the genes and enzymes involved in determining the composition of corn oil.

D. Interaction in the Accumulation of Kernel Components

The accumulation of starch, protein, and lipids during kernel development is not totally independent. The correlated effects of various mutants and mutant combinations have been the subject of numerous studies.[306–309] Notably, the mutants affecting kernel carbohydrates also reduce the amount of zein accumulated in the kernel.[25,306] Furthermore, the reduction of zein content was further enhanced in double mutants of *o2* and the starch mutants, with kernels of the double mutants having less zein than kernels of either single mutant. However, kernels of the double mutants *bt2 fl2* and *sh2 fl2* have the same amount of zein as kernels of the single mutants *bt2* and *sh2*. Conversely, *o2* kernels have reduced levels of starch as well as a major reduction in zein content.[310] The mutants affecting kernel proteins and carbohydrates alter the osmotic potential of the immature kernels in a similar fashion. The accumulation of free amino acids in the protein mutants and sugars in carbohydrate mutants increases the osmotic potential of the endosperm. Under these conditions, water flow into the kernel is favored, but movement of osmotically active sugars and amino acids would be expected to be reduced. Thus, correlated reduction in the synthesis of starch or protein would occur. Conversely, the oil percentage of the kernels of protein and carbohydrate mutants is higher. For example, *bt2*, *fl2,* and *su2* kernels have increased oil. However, most of this difference can be explained by a greater reduction in endosperm size than embryo size in these mutants.[311–313]

V. GENE CLONING AND BIOTECHNOLOGY

Many of the mutants discussed in this chapter have been cloned utilizing various techniques (Table 1.5). In addition, the genes encoding many of the enzymes of the reactions of carbohydrate metabolism and the structural genes for storage proteins have been cloned. Both cDNA and genomic clones have been isolated and characterized for most genes. Analysis of the transcribed regions of the genes and the upstream regions of these genes has identified promoters and enhancer sequences. Various combinations of promoters and enhancers with the gene of interest will allow the expression of these chimaric genes in a tissue-specific or temporal-specific manner. It is easy to envision the benefits of modulating gene expression in two ways: either elevating gene expression or eliminating gene expression. However, in other instances, the end product may require reduced gene expression and, depending upon the objective, varying levels of expression may be needed for the production of the desired phenotype at the whole kernel or biochemical level. For example, an ideal sweet corn cultivar would be one that maintained a high sugar level during the desired eating stage, but that accumulated sufficient starch late in kernel development to provide higher seed quality. This could be achieved by the temporal control of the developmental expression as well as modifying the levels of expression of various genes.

The potential of manipulation of starch, protein, and other biochemical properties of corn and other crops is clear.[321,322] The potential of the use of genetic engineering for the production of novel variation has recently been demonstrated in the carbohydrates of potato tubers. The development of potato lines with amylose-free starch (*waxy*) and low starch (reduced ADPG pyrophosphorylase) tubers has recently occurred.[323,324] These studies utilized antisense RNA constructs to reduce gene expression. The amylose-free potatoes have also been transformed with an active

TABLE 1.5
Cloned Genes Involved in Carbohydrate Metabolism of Storage Protein Accumulation in Corn Kernels

Gene	Biochemical Function	References
Carbohydrate Metabolism		
sh1	Sucrose synthase (endosperm specific)	89,91
Sus	Constitutive sucrose synthase	159,160
Sh2	ADP-Glc pyrophosphorylase (subunit)	157
bt1	Adenylate translocator	94
bt2	ADP-Glc pyrophosphorylase (KDA subunit)	155,156
du1	Starch synthase II	198
wx1	Starch granule bound starch synthase	93,314
su1	Isoamylase	193
ZPU1	Pullulanase	190,192
BEI	Branching enzyme I	316
ae	Branching enzyme II	317
Protein Metabolism	**Regulatory protein**	
o2	Leucine-zipper	92,315
Glb 1	Major globulin protein (63 KD)	318
Glb 2	Major globulin protein (45 KD)	319
glutelin-2	Glutelin-2 gene (28 KD)	320
Zp1, Zp2, etc.	Zein protein	261,262,264,265,269

starch granule-bound starch synthase gene.[325] The tubers of these plants contain varying levels of amylose. In order to successfully use similar methods in corn, reliable methods for genetic transformation need to be developed. Recent reports by several groups have reported methods to transform corn using different techniques.[326–329] These developments provide completely new methods for the production of genetic variation for specialty corns.

VI. FUTURE PERSPECTIVE

Corn is clearly a diverse crop with many specialty uses and types. These types have evolved from a rich past of selection based on recognition of unique properties associated with various genetic variants. The continued analysis of genetic variation has provided additional resources for the refinement and development of specialty corn. The ability of the biotechnologist to manipulate genetic variation at the level of specific genes offers the potential to tailor genetic variation for the production of precisely designed specialty corn in the future. This process, by necessity, will go beyond the biotechnologist. It is clear that the design of specialty corns in the future will require the expertise of the biotechnologist, breeder, biochemist, and product specialists.

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2 Properties of Corn Starch

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I. INTRODUCTION

Starch is the reserve carbohydrate in the plant kingdom, existing as tiny granules deposited in the seeds, tubers, or roots of plants. Although starch occurs throughout the plant world, only a few plants are used to produce it commercially, and corn (*Zea mays* L.) is the major source of starch produced worldwide.[1] Starches from potato (*Solanum tuberosum* L.), wheat (*Triticum aestivum* L.), and to a lesser extent, rice (*Oryza sativa* L.) also have worldwide markets. In the U.S., 95% of the starch manufactured is from corn. Details on the processing of corn starch may be found elsewhere.[2,3]

Typically, starch yield from wet-milling corn is 66% on a dry-weight basis, calculated as a percentage of the raw corn, making starch recovery an economical process.[3] These figures refer to wet-milling of normal dent corn, which is the predominant corn processed for starch. The yield from wet milling *waxy* (*wx*) corn is 90%, and from high-amylose (60 to 70% amylose) just 80% to 90% of the expected yield from dent corn.

Starch is stored in granules as two major types of polymers referred to as amylose and amylopectin. Amylose is essentially linear, with anhydroglucose molecules linked through alpha-*D*-(1-4) glucosidic bonds and with some minor branching at carbon 6 by alpha-*D*-(1-6) glucosidic bonds. Amylopectin also contains anhydroglucose molecules linked through alpha-*D*-(1-4) glucosidic bonds, but it contains many periodic branches at the carbon-6 position. In corn and some other plants, there is evidence of starch molecules intermediate in size (molecular weight) to amylose and amylopectin.

The intent of this chapter is to discuss corn-starch properties in normal and mutant corn by describing molecular structure; granular organization; thermal, mechanical, physical, and functional properties; and uses of normal and some specialty corn starches.

II. HISTORY AND FUTURE OF CORN STARCH PRODUCTION

A. Early History

Corn is thought to have originated in Mexico, moving northward to Canada and southward to Argentina.[4] Evidence of corn production has been traced as far back as 5000 B.C., when it was already a domesticated crop, relying upon humans for its survival.

Most modern corn types are derived from early races developed by native Mexicans and Central and South Americans. Separate races of corn were maintained for production of foods, beverages, and medicines because of their individual properties. It is likely that some of these unique properties were caused by differences in the starch composition and structure. Yellow dent corn produced as the major corn crop in the farm belts of the U.S., Canada, and in much of Europe was developed from races produced in postcolonial North America. It is the common starch from these "Corn Belt dent hybrids" that has been studied almost exclusively, and that is produced in bulk in industries in the U.S.

B. American History

Corn starch was first produced in the U.S. in 1844 at a plant owned by William Colgate in Jersey City, New Jersey.[5] The production followed the development by Thomas Kingsford, of a process for corn-starch purification by alkaline treatment. After the development of this simplified process for corn-starch manufacture, many U.S. starch plants began converting their operations from wheat-starch to corn-starch utilization, including a plant in Utica, New York, in 1849 and a plant in Cincinnati, Ohio, in 1854. By 1895, there were 16 corn-starch processing plants operating in the U.S., producing 90.7 million kg of starch per year. By the late 1980s there were 10 American companies and 28 wet-milling plants having corn-starch-producing capabilities.[3,5]

C. Corn Starch Uses and Future Outlook

Although the majority of U.S. corn production (62%) is used as animal feed, an important amount (19.4%) is used for food, seed, and industrial purposes.[6] In particular, the starch derived from corn provides the basis for an important industry. Corn starch is used as a food ingredient, either in its native form or chemically modified, to thicken liquids and to contribute other desirable textural properties to foods. About 8.4% of the 1997/98 U.S. corn crop (year ending August 31, 1998) was milled into sweeteners such as corn syrup, high-fructose corn syrup, and corn sugars, representing a continuous recent rise in sweetener production and consumption.[7] Indeed, a striking change in U.S. sugar consumption occurred over the past two decades. Sucrose's share of total caloric sweetener use decreased from 83% in 1970 to 44% in 1996, whereas the share from corn sweeteners increased from 16 to 55% during that same time period.[8] Corn starch also can be fermented to alcohol, including fuel ethanol.[9] The paper industry is the largest nonfood user of native and modified starches. Examples of other nonfood uses for corn starch or unpurified corn flour include use as binders in gypsum board and other building materials, in adhesives, and in the development of thermoplastics and polyurethanes.[9,10]

III. GENETICS OF STARCH DEVELOPMENT IN CORN

The focus of this section is to give a brief review of the genetics of corn-starch development to aid in further discussion of properties of different corn starches. For detailed information on corn-starch development, several complete sources can be consulted.[11–13]

In normal corn kernels, basal endosperm cells begin synthesizing starch late in kernel development.[14] This reserve starch accumulates during kernel maturation as starch granules within the cells, increasing from less than 10% of kernel weight at 7 to 10 days after pollination to 55 to 60% of kernel weight by 30 to 35 days after pollination, remaining fairly constant from then until maturity.[15,16]

Starch granule formation occurs along two gradients: a major gradient from the crown to the base of the endosperm and a minor gradient from the crown out to the periphery. Thus, at any one time, cell maturity differs among the endosperm cells. Within an endosperm cell type, all mature starch granules are of similar size; the younger the granule, however, the smaller its size and the lower the apparent amylose percentage.[17–20] Some starch types exhibited differences in thermal properties, such as increased gelatinization onset temperature, at 12 days after pollination compared with later dates, suggesting structural differences in starch based on kernel maturity.[21] Therefore, there also may be some differences in starch composition among the cells of a single plant or kernel, based on many factors.

Planting date also affected thermal starch properties, with later planting dates significantly ($p < 0.05$) increasing peak temperature and enthalpy of gelatinization.[22] Later planting dates may be associated with higher growing temperature (35°C vs. 25°C), which previously was shown to reduce grain yield, kernel weight, kernel density, starch granule size, percentage of apparent amylose, and amylose molecular size, and to increase gelatinization temperature.[23] Ng et al.[21] noted that starches from 62 lines of a high-yielding exotic corn population, Antigua 1, had significantly greater ($p < 0.05$) values for several thermal properties, including peak onset and enthalpy of gelatinization, when grown in Georgia rather than Puerto Rico. Again, increased temperatures during grain filling in Georgia likely contributed to these results. Even pollination method affected starch thermal properties.[24]

IV. MOLECULAR STRUCTURE OF CORN STARCHES

A. Dent (Normal) Corn-Starch Composition

1. General Starch Composition

The behavior of starches in products is related to its structure. Chemically, corn starch is composed of anhydroglucose units, organized into two structurally distinct molecules, amylose and amylopectin, and some intermediate materials that retain characteristics of both the major fractions. Some information about the distribution of these fractions within a starch type can be obtained through basic chemical tests on the whole starch fraction, which has been isolated and purified from the plant as described elsewhere.[25,26] For example, amylose and amylopectin have different iodine-binding properties, with corn amylose and amylopectin giving iodine affinity (IA) values of about 19 to 20% and 1%, respectively, depending upon the source and genetic background. The amount of "apparent amylose" can be determined either by measuring the absorbance of the starch-iodine complex and relating this value to that of pure amylose and amylopectin standards[12] or by measuring the amount of iodine (mg) bound per 100 mg of starch in a potentiometric titration and relating the value to the amount bound by an amylose standard.[27,28] Values based on iodine binding, however, are only estimates of amylose content because of differences in the binding abilities (and structure) of amylose and amylopectin among starch types. For example, amylopectin molecules with long external branches bind more iodine than do those with short branches.[29,30] Likewise, short-chain amylose molecules bind less iodine than do longer chain amyloses.[30] The wavelength at which a starch–iodine complex has maximum absorbance is referred to as the lambda max, and the value at that wavelength is called the blue value (BV).

To study the specific properties of the starch components and to get a quantitative measure of the amount of amylose in a starch, the starch must be fractionated by precipitation and/or by molecular weight (MW) into its individual components.[31] By applying specific procedures, many important characteristics about the fractions can then be determined, including IA, BV, average chain length (CL), degree of polymerization [number (DPN) or weight (DPW) average], and MW distribution. These procedures also can be used on the whole starch for general overall values. The average CL refers to the number of glucose residues per nonreducing end-group, and the DP represents the number (or weight) of glucose residues per reducing group. A nonreducing end-group occurs on the last glucose molecule in a macromolecule chain linked by alpha 1-4, and possibly alpha 1-6, glucosidic bonds; its first carbon may be bonded, and is thus incapable of reducing an alkaline solution of cupric ion in a chemical test. In contrast, a reducing group contains a free alpha-OH group that occurs on carbon 1 of a glucose unit that is not further linked and is, thus, capable of reacting chemically as described. It follows that in completely linear molecules, CL = DP; in highly branched molecules such as amylopectin, however, DP >>> CL.

2. Fine Structure of Amylose

As described earlier, amylose is essentially linear, with the anhydroglucose units predominantly linked through alpha-*D*-(1-4) glucosidic bonds. A summary of the properties of corn amylose is presented in Table 2.1. Originally, it was thought that amylose contained no branch points, but evidence now exists to prove that amylose consists of a mixture of linear molecules and molecules with limited, long-chain branching involving alpha-(1-6) linkages.[25,32,33] The MW of amylose is generally around 50,000 to 200,000, but MWs of up to 1,000,000 have been reported.[34] The MW of corn amylose typically ranges from 100,000 to 200,000.[35]

The amylose concentration in normal corn averages about 25%, with the remainder being amylopectin and the intermediate materials fraction. But these percentages vary among cultivars and during kernel development, as previously described. For example, amylose percentage ranged from 20 to 36% for 399 cultivars of normal corn.[36,37] Although starch composition of some plant

TABLE 2.1
Properties of Amylose and Amylopectin from Normal Corn

Property	Amylose	Amylopectin	Ref
Molecular weight	1–2 × 10^5	> 2 × 10^7	35
Degree of polymerization (DPN — number of glucose residues)	990	7200	32[a]
Glycosidic linkages	Mainly α-D-(1 → 4)	α-D-(1 → 4), α-D-(1 → 6)	
Molecular shape	Essentially linear	Very branched	
Susceptibility to retrogradation	Great	Little	
Lambda max of iodine complex	644 nm	554 nm	32[a]
Iodine affinity	20.1 g/100 g	1.1 g/100 g	32[a]

[a] Values are for commercial corn starch.

species varies with growing conditions associated with different locations, years, planting dates, etc., little variation in amylose percentage was seen in normal maize starch from plants grown for 3 years in eight different states.[38]

Takeda and co-workers[32] made great strides in elucidating the molecular structure of corn-starch fractions from commercial and several laboratory-prepared corn starches, presumably originating from regular dent corn. The method of starch preparation had little impact on their results. The amyloses were small molecules with DPN = 930 to 960, and DPW = 2,270 to 2,550. The DPN value of the corn amylose was similar to that of rice amylose (920 to 1,110),[39] but less than that of amylose from wheat (1,290), kuzu (1,540), chestnut (1,690), nagaimo (2,000), lily (2,310), tapioca (2,660),[40] sweet potato (3,280 to 4,400),[41] and potato (4,920,[42] 6,340[43]). Similarly, corn amylose had the smallest DPW value among the same starches. The CL of the corn amylose (295) was similar to those of wheat (270), rice, kuzu, sweet potato, and tapioca (310 to 340),[40] but less than those of chestnut (375),[44] lily (475), nagaimo (535),[40] and potato (670).[42] Further, corn amylose had the least chains per molecule among these starches. There were nearly equal numbers of branched and unbranched molecules in the amylose fraction, and the branched portion had an average of about five branch points per molecule. In general, the amyloses were made up of large branched molecules and small unbranched molecules, similar to rice and wheat amyloses,[40] and were similar to amyloses from other botanical sources in IA, BV, and lambda max.

3. Fine Structure of Amylopectin

Amylopectin is a macromolecule containing both (1-4)- and (1-6)-alpha-*D*-glucosidic linkages to form a ramified structure. Table 2.1 provides a summary of properties of corn amylopectin. In general, this molecule ranges from MWs of 1,000,000 to 3,000,000, but some are as large as 5,000,000.[25,34] Corn amylopectin was estimated to have a MW of more than 2,000,000.[42] As early as 1956, it was proposed that amylopectin contained three different types of chains.[45] Although the structure of amylopectin has been studied extensively with techniques, such as enzyme debranching and gel permeation chromatography (GPC), the exact arrangement of the chains within the amylopectin molecule is still not clear. Among the several models proposed for the structure of amylopectin, the cluster-type model is most widely accepted. Researchers proposing cluster-type models or modifications of previously proposed models include Nikuni,[46] Whelan,[47] French,[48] Robin et al.,[34] and Manners and Matheson.[49] Briefly, each macromolecule has one C chain, which carries the only reducing group. The B chains are linked to the macromolecule by their reducing group and contain one or more A chains that are similarly linked. The B chains are further categorized into B1, B2, and B3 chains, according to the number of crystalline regions through which they pass. The ratio of A to B chains is a measure of the degree of multiple branching and is an important

property describing amylopectin. Many early measurements of the A- to B-chain ratio in amylopectin were incorrect because of an improper concentration of isoamylase used in the procedure. It is now generally agreed that the A- to B-chain ratio ranges from 1:1 to 1.5:1, and this arrangement fits nicely within the cluster models that have been proposed.[50]

Several studies using debranching enzymes have shown that the CL of amylopections of various origins are slightly different.[51–56] Takeda et al.[32] evaluated the structure of amylopectin fractionated from regular corn starch. The amylopectin had a relatively high IA (1.10 g/100 g), ranging between those of japonica rice (0.37 to 0.89 g/100 g) and indica rice (1.62 to 2.62 g/100 g) amylopectins.[39] The DPN value for the commercial starch was 7200. The CL value was 21.4; greater than those of *wx* corn (18.6)[57] and japonica rice (19–20)[39] amylopectins, similar to those of indica rice (21–22),[39] kuzu (21.1),[58] and sweet potato (21–22)[41] amylopectins, and less than those of lily (23.6),[59] potato (23.7),[58] and nagaimo (24.0)[60] amylopectins. Upon fractionation, the corn amylopectin had a large number of long chains, which, along with the high IA, suggested that the amylopectin was composed of long outer chains with widely spaced side chains, similar to rice amylopectin.[39]

4. Intermediate Materials Starch Fraction in Corn

Both amylose and amylopectin can be further subfractionated into a graded series of molecular sizes, making the study of starch a very complicated matter. In addition to the heterogeneity present in these fractions, there also is evidence of intermediate materials in corn and other starches. The intermediate materials fraction contains chains of (1-4)-linked alpha-*D*-anhydroglucose residues, but the average length of these chains and the number of chains per molecule are different from those in either amylopectin or amylose. Boyer et al.[61] and Yeh et al.[62] demonstrated the presence in normal corn starch of about 5% to 7% intermediate materials, basing their conclusions on indirect evidence from IA.[63] A similar estimate of 7% intermediate materials in corn starch was given based on butanol precipitation.[64] The amounts of intermediate materials can be quite high in starch from some mutant corn genotypes, as discussed later in this chapter.

B. Mutant Corn Starch Composition

1. Structure of Starch from High-Amylose Corn Mutants

a. Introduction

A measurement of the amylose and amylopectin contents of a corn starch gives only partial information about its properties. Although fine structural differences among normal dent corn starches seem to be minimal, the introduction of genetically variable corn or of corn mutants can produce many unique starch structures. Additionally, various gene combinations can give the same amylose content but may introduce differences in the fine structure of its molecular components. Scientists have only begun to uncover the effects on starch structure and properties of these many mutant combinations and of the interaction with background genotype.

It is generally accepted that starch granules from high-amylose corn are more compact and more crystalline than those from normal corn and that they will not easily disperse into solution without some form of pretreatment.[65] This behavior has made fractionation of these starches very difficult, but gel permeation techniques have helped in the molecular study of these and other starches.

b. Amylose Component of High-Amylose Corn

The recessive amylose-extender mutant gene (*ae*) causes an increase in the apparent amylose percentage of the corn starch. There is great variability in the amount of apparent amylose in the starch from plants containing the *ae* gene, depending upon the corn inbred with which it is crossed,[66–69] with a reported range of 36.5 to 64.9%.[67] Minor modifying genes in the background corn inbred are thought to account for apparent amylose concentrations of 50 to 70% in a series

of hybrids developed with *ae* for their consistent high-amylose contents.[28] A U.S. patent was obtained in 1994 for discovery of a "low amylopectin" starch from corn, which contained at least 75% amylose, but optimally at least 85% amylose. The starch was obtained from corn having a recessive *ae* genotype coupled with numerous amylose extender modifier genes.[70]

Takeda et al.[71] examined the molecular structures of three varieties of amylomaize amyloses. These included a laboratory-made amylomaize and two commercial starches, Hylon V and Hylon VII (starches from National Starch and Chemical Co., Bridgewater, N.J.), with apparent amylose contents, as calculated by the IA, of 54, 48, and 68%, respectively. The amyloses showed normal iodine-staining properties, similar to other amyloses,[32,40] but had slightly lower IA values than those of amylomaize amyloses reported elsewhere.[72] The DPN and DPW of the three amyloses were less than those of normal maize amylose.[32] Thus, the amylomaize amyloses were composed of smaller molecules than in normal corn amylose, as also reported elsewhere.[72] Greenwood and MacKenzie[73] examined the properties of Amylon 50 and Amylon 70, amylomaize starches reported to contain 50% and 70% of amylose, respectively, but whose IA corresponded to only 38% and 45% of amylose, respectively. Although the amylose fractions were impure, it was concluded that the amyloses were of relatively short chain length.[74] The amylomaize amylose CL values (215 to 255) also were lower than those of normal corn (295).[40] The DPN/CL ratios were similar for the three amylomaize and normal amylose molecules, indicating an average of three chains per molecule.

The amylomaize amyloses had nearly equal numbers of large branched molecules and small unbranched molecules, with the branched molecules having five or six chains on average. The branched molecules present in the three amylomaize amyloses were similar and showed slightly lower IA, BV, and lambda max than those in the parent amyloses, a characteristic also found in normal amyloses. But the amylomaize amyloses had smaller branched molecules, with a narrower MW distribution than did normal maize amylose. Klucinec and Thompson,[64] however, determined that the amylose fractions from normal corn starch and from Hylon V and Hylon VII all had similar size distributions and iodine-binding behavior.

Recently, a dominant mutant allele of the *ae1* locus was reported,[75] thus allowing accelerated development of high-amylose inbred and hybrid lines.[76] Corn plants homozygous for the dominant amylose extender allele, *Ae1*-5180, and crossed with wild-type *Ae* plants resulting in zero to three doses of the *Ae*-5180 allele were evaluated for their starch endosperm.[77] The apparent amylose contents of the starches from dominant *Ae* corn ranged from 54 to 58%, but did not significantly differ with *Ae* gene dose from 1 to 3. The "real" amylose content, calculated after subtracting BV readings from the amylopectin and intermediate fractions, averaged about 33%.

c. Amylopectin Component of High-Amylose Corn

The nature and structure of the branched component of amylomaize starch has caused considerable interest. Greenwood and MacKenzie,[73] in an attempt to fractionate the amylopectin portion of Amylon 50 and Amylon 70 amylomaizes, found one fraction with a CL of 28 units, comparable to that for normal amylopectin, and another branched fraction having an apparent CL of 36 units. They, however, suggested the presence of contaminating short-chain amyloses in the latter fraction.

Previous work also had shown a high average CL of 36 glucose residues in the branched-chain portion of amylomaize starch.[65] With GPC, Mercier[78] found amylopectins from amylomaize (64% amylose) to have longer inner chains (CL about 60) than those from *wx* or normal starches (CL = 30). Yamada and Taki[79] and Inouchi et al.[53] also confirmed these qualitative differences between normal and amylomaize amylopectin fractions by use of GPC. In a study on the effects of gene dosage at the *ae* locus of maize on the amylopectin fraction of starch, Boyer et al.[80] found high-amylose starches to contain the altered amylopectin observed by others. Baba and Arai[81] determined that amylomaize amylopectin had a CL approximately 10 glucose units longer than that of waxy-maize amylopectin, and that the difference was a result of the relative amounts of two fractions. The CL of the internal chains in amylomaize amylopectin was nine glucose units longer than in

waxy-maize amylopectin, and outer branches were less frequently branched. Thus, these data confirmed that amylomaize amylopectin had longer inner and outer branches than did normal amylopectin. Yum and Matheson[82] further showed a decrease in molecular size and an increase in average chain length in the amylopectin from *wx,* normal, and *ae,* in that order.

Takeda et al.[83] compared the amylopectin fractions from three different high-amylose corn starches, including Hylon V (36% apparent amylose), a laboratory-prepared sample (41% apparent amylose), and Hylon VII (59% apparent amylose). They separated the amylopectin into four fractions, based on MW. The greatest MW fraction was present in relatively low quantities and had an IA of 1.94 to 2.50 and an average CL of 29 to 32 for the three types of starch. Both values increased with decreased MW of the fractions to an IA of up to 7.23 and CL of up to 41. In contrast, normal corn starch amylopectin had a CL of about 20 to 21.9. The percentage of long side-chains increased with a decreased MW; therefore, the lowest MW fraction had the greatest IA and CL (more like amylose). The Hylon VII had a great amount (27%) of the short-chain component. In general, they found a wide distribution of MW in the amylopectin fractions. Klucinec and Thompson[64] also found highly branched amylopectin with a broad MW distribution in Hylon V and Hylon VII starches.

The amylopectin of the *Ae1*-5180 dominant starch contained long branch-chain lengths of about 45 glucose units for the long (B2) chains and about 19.1 glucose units for the short B and A chains when measured by GPC.[77] The A to B ratio was 1:1.7, similar to the pattern obtained for amylopectin of *aebt1*.[33] Amylopectin branch chain lengths for the *Ae1*-5180 starch were shorter than for the high-amylose V and VII starches.[33,84] Amylopectin from native normal maize starch by this GPC method had long (B2) chain lengths of about 42 glucose units, short B and A chains of about 14 glucose units, and an A to B ratio of 1:3.0.[33]

d. Intermediate Component of Starch from High-Amylose Corn

The amylomaize starches are reported to contain a large portion (up to 15%) of intermediate components, which, along with the presence of long-chain amylopectin, account for discrepancies between iodine-binding estimates of amylose content and other measures of the amylose percentage.[30, 85,86] The average degree of polymerization of this material was estimated to be 250 to 300 glucose units per molecule, and the average CL of the molecule was about 50. Thus, the intermediate fraction was a branched polysaccharide of low MW. Baba and Arai[81] suggested its structure was four or five branches with CL of about 50, linked to a main linear chain of 100 to 150 glucose units.

The *Ae*1-5180 dominant starch also had a high proportion of intermediate materials (percentage not given), with fairly long branches, similar to the amylopectin.[77] Long branch chains (B2) of this intermediate fraction contained about 52 glucose units, whereas the short B and A chains were about 21 glucose units in length. The A to B ratio was 1:1.7. There were no B3 chains in the intermediate component.

2. Structure of Starch from Waxy Corn Mutants

The *waxy* (*wx*) mutant produces an endosperm starch granule containing nearly 100% amylopectin. In general, amylopectin molecules from different species vary in chain length[87,88] but stain red rather than blue with the addition of iodine, giving no apparent amylose content. Furthermore, the *wx* mutant is epistatic to all other known mutants in its resulting lack of amylose accumulation.[11,62] The starch molecules in some corn genotypes containing the *wx* gene are loosely branched, with long external chains, resulting in some binding with iodine and in a small measure of apparent amylose.[79,89,90] Chromatographic profiles of *wx*-containing starches, however, revealed no amylose peak.[62,79,89,91]

Manners and Matheson[49] reported an A to B chain-length ratio of 1:1 in the amylopectin of *wx* corn starch. Elsewhere, GPC of the debranched products revealed distribution patterns of branch

lengths that were similar to the amylopectins from *wx* corn, *wx* rice, and potato amylopectin.[92] In each instance, two peaks were seen, one for a CL of 50 and one for CL 20. Further, it was suggested that, in amylopectin, the B chains are divided into longer (CL = 40 to 80) and shorter (CL = 20 to 40) chains. By using the same technique, Mercier[78] found amylopectins from *wx* and normal starches to be identical. Yamada and Taki,[79] however, found amylopectin from normal starch to have a lambda max of 550 nm, whereas that from *wx* starch was 530 nm. Jane et al.[93] reported branch chain lengths in *wx* corn starch of dp 23 to 29.

3. Structure of Starch from Other Corn Endosperm Mutants

Other corn mutants known to affect the structure and the amylose content of starch, in addition to those of *ae* and *wx,* include *sugary-1* (*su1*), *sugary-2* (*su2*), and *dull* (*du*). The effects of *su1, su2,* and *du* on apparent amylose percentage are not as dramatic as the effects of *ae* and *wx,* so historically they have not been developed into specialty corn crops to be used for their starch production, as were *ae* and *wx* types. Early work[94–96] measuring the apparent amylose content of these single mutants and of their mutant combinations showed only slight differences in the amylose percentages among the starches: 29 to 33% for *su* (presumably *su1*), 28 to 49% for *su2,* and 34 to 38% for *du*. With the development of more sophisticated techniques for measuring the starch fractions, such as GPC, researchers have reevaluated starch components in corn mutants and in their double mutant combinations. Yeh et al.[62] reported the effects of *ae*, *du*, *su (su1?)*, and *wx*, alone or in multiple combinations, on the amylose percentage as measured by GPC of the native starch (Table 2.2). By their measurements, the amylose contents were 65% for *su* and 55% for *du*, a greater contrast to the values reported previously. Wang et al.,[33] however, obtained lower amylose percentages of 31.2% for *su1* and 30.5% for *du1*, by GPC of the debranched starch. When the native (unbranched) starches were fractionated by GPC, amylose percentages of 40.5% for *su1* and 45.5% for *du1* were measured, seemingly because intermediate materials were included in this analysis.

By using GPC, Ikawa et al.[86] confirmed the high amylose contents of starches isolated from *su1* and *du* mutants and also reported a high amylose content for *su2* starch. As noted earlier, Ikawa et al. found large discrepancies in amylose contents for starches of *ae* as measured by their GPC procedure compared with potentiometric IA values reported in the literature. The same situation was suggested for starches from *aewx*, *aesu1*, and *aesu2*. The estimation of amylose by the IA procedure is inaccurate because the presence of branched components with long external chains results in an overestimation of the amylose content or the presence of short-chain-length amylose causes the amylose content to be underestimated.[22]

In studies of endosperm mutants and their double-mutant combinations with *opaque-2* (*o2*) and *floury-2* (*fl2*) genes, Barbosa and Glover[97] demonstrated no substantial effect on the ratio of amylose to amylopectin for *o2* and *fl2* double-mutant combinations with other endosperm mutants, except for the *su1o2* combination, which showed decreased amounts of amylose. The *su1o2* combination, however, had an increased amylose content compared with the *su2* counterpart.

Ikawa et al.[86] found a normal-type amylopectin in *su2*, whereas a novel-type amylopectin was noted in *su1* and *du* starches. Inouchi et al.[98] also found little difference in the fine structure of amylopectin from *su2* and normal corn starches based on unit chain length distributions. In *su2* amylopectin (in an Oh43 background), however, Takeda and Preiss[99] measured larger-sized long B chains, which were poorly branched, thus leading to increased iodine affinity values. Amylopectin of *su2o2* was similar to that of normal, and those of *su1o2* and *duo2* were a novel type possessing 62% or less of the longer branches. Only small amounts of the amylose fraction were apparent in the *aewx*, *wxsu1, wxsu2*, and *duwx* mutant starches, demonstrating the epistatic nature of the *wx* gene for amylose production. Combination of the *ae* gene with the other starch-modifying genes, except for *du*, increased the contents of amylose and the intermediate fractions and increased the chain lengths of amylopectin. Presence of the *ae* gene also reduced the susceptibility of the starch granules to amylase attack. The *du* gene seemed to be epistatic to the *ae* gene for some

TABLE 2.2
Amylose Percentage of Starch from Corn Genotypes Determined by Using Gel Pemeation Chromatorgraphy

Source of Starch[a]	Amylose[b] (%)	Amylose[c] (%)
Normal	27	29
ae	46	33
du	31	55
su	31	65
wx	0	0
ae du	57	47
ae su		28
ae wx		0
du su	35	70
du wx	0 (*wx du*)	0
su wx		0
ae du su		31
ae du wx		0
ae su wx		0
du su wx		0
ae du su wx		0

[a] *ae* = amylose extender, *du* = dull, *su* = sugary, *wx* = waxy, *bt* = brittle, *h* = horny, *sh* = shrunken.
[b] Data from Wang et al.[33]
[c] Data from Yeh et al.[62]

properties, resisting the increase in the amount of intermediate fraction and in the chain length of amylopectin.

Although the amylopectin of *su1o2* was a different type from that of *su2o2*, the double-mutant combinations of *su1* and *su2* with *wx*, *du*, and *ae* did not result in these differences. For example, the pairs, *wxsu1* and *wxsu2*, *dusu1* and *dusu2*, and *aesu1* and *aesu2,* had similar starch components. Therefore, the *su1* and *su2* genes seemed to be more easily modified by the *wx*, *du*, and *ae* genes than by the *o2* gene.

The double-mutant combinations *dusu1* and *dusu2* produced increases in amylose contents, to 61.5% and 59.1%, respectively, compared with amylose contents of 35.6% in *duo2*, 42.2% in *su1o2*, and 39.2% in *su2o2*. In contrast to the synergistic effect of *du* to either *su1* or *su2* for amylose production, no enhanced effect was observed when *su1* was combined with *su2*, suggesting a different mechanism of amylose production for the *du* mutant than for those of *su1* and *su2*.

Boyer et al.[89] studied the nature of the effect of the *ae* mutant on amylopectin structure by examining the fine structure of *aewx* starch. The starch contained 21% apparent amylose and had a lambda max of 580 for the iodine-starch complex. By using GPC techniques, they determined that the *aewx* starch was loosely branched with an average internal CL of 52 glucose units, compared with a length of 30 glucose units for *wx*. The *aewx* outer chains were longer than those of *wx* and fewer in number per weight of starch. In general, the *aewx* starch had a unique structure that was similar to the anomalous amylopectin (intermediate fraction) reported in *ae* starch. Later, Boyer et al.[80] systematically studied the effect of gene dosage at the *ae* locus of maize on the amylopectin fraction of the endosperm starch. In general, increased doses of the recessive allele resulted in amylopectin fractions with iodine spectra having absorption maxima at higher wavelengths and greater absorptivity than in the original starch, which agreed with earlier work.[89] Starches from endosperm of different *ae* dosage, but homozygous *wx*, contained no amylose by GPC and had increased average CL with increased *ae* dosage. In addition, increased dosage at the *ae* locus,

regardless of the genotype at the *wx* locus, resulted in amylopectin with increased linearity, again in agreement with their earlier work.[89] Short-chained amylose (approximately 100 glucose units) was observed in all *ae* genotypes in a homozygous *Wx* background.

Yamada et al.[90] confirmed this unique structure of starch from *aewx* corn, which they termed "amylo-waxy" corn. They further demonstrated differences in the starch structure, depending upon which gene, *ae* or *wx*, was dominant. It was clearly observed, however, that hidden *ae* extender genes also participated in the starch production.

The CL distribution of amylopectins in the starch of several single mutants and of their normal counterparts in an Oh43 background were compared by using GPC.[98] The *su1* intermediate fraction also was examined. The ratio of A to B chains for the *ae* starch seemed to be high and that for *su1* intermediate materials was low, with no long B chains. The ratio of short B to long B chains for the *du* starch was high and that for the *ae* starch was low. The unit CL distributions of amylopectins for the normal, *wx*, and *su2* starches were similar.

Wang et al.[33] characterized corn starches from 17 endosperm mutant genotypes (single and double mutant combinations) in a common Oh43 inbred background. In general, interactions between recessive mutant genes influenced the starch structure and granule morphology (size and shape) of the different genotypes. As noted earlier, the *ae, du1, and su1* genes were associated with increased amounts of amylose and intermediate materials compared with normal starch. The proportions of long B chains and the average chain length of amylopectins were increased when the *ae* gene was present. In contrast, the *du1* gene decreased the proportions of the long B chains of amylopectins. The mutants containing the *ae* gene showed low degrees of branching in the amylopectin; mutants containing the *du1* and/or *su1* genes had high degrees of amylopectin branching. These findings were confirmed by detailed analyses of the amylopectin and intermediate materials of these mutants.[100]

Fuwa et al.[101] examined CL distribution of amylopectins of double- and triple-mutants containing the *wx* gene in the inbred Oh43 background. Amylopectin of the *aewx* mutant had an increased proportion of long B chains and decreased proportion of short B chains compared with *wx* amylopectin, whereas amylopectin of the *duwx* mutant had a decreased proportion of long B chains and an increased proportion of short B chains, thus confirming the novel nature of the *aewx* and *duwx* amylopectins. The A to B chain ratios, however, for amylopectins from *aewx*, *aewxfl2*, *aewxsu1*, *aewxsu2*, *btwx*, *duwx*, and *su2wx* were in the range of 1.1 to 1.4 and were all similar to that of *wx* amylopectin.

The isolation and characterization of starches of *su1*, *brittle-1* (*bt1*), *su1bt1*, and the normal corn counterpart by using GPC showed a higher amylose content of 43% for *su1bt1*.[102]

As if all the variations in starch structure with mutant introductions were not enough, there also is evidence that genetic background influences starch properties. Boyer and Liu[103] studied nonmutant and single-, double-, and triple-mutant combinations of the endosperm genes *ae*, *du*, *su*, and *wx* in four corn inbred lines. Minor, yet predictable, effects of endosperm genes on starch properties were noted. For example, starches from *wx* endosperms contained no amylose, and the mutants *ae*, *du*, and *su* produced starches with increased amylose contents. The production of amylose in mutant endosperms was greater in corn having dent inbred background, followed by sweet-corn inbreds, but the production of low-MW amylopectin and intermediate materials fractions was greatest in a corn having sweet-corn inbred background. Thus, the mutant genes exerted a predictable effect, but the magnitude of the effect differed, depending upon the genetic background material.

Clearly, there are many factors affecting starch structure in corn, and scientists are only beginning to discover the magnitude of possibilities for manipulating these structures and the effects these changes could have on the functional properties of the starch. The evolution of newer techniques for studying starch structure also will enhance the development of new starches. High-performance size-exclusion chromatography (HPSEC) to separate starch components by MW, along with several possible detection systems, is very promising. Pulsed amperometric detection (PAD)

allows a direct measure of carbohydrates at alkaline pH and has been useful in showing chain-length distributions of debranched amylopectin.[93] Single- and multi- angle laser light scattering (SALLS and MALLS, respectively) offer the ability to observe absolute MW of the starch, whereas viscometry detection measures intrinsic viscosity or molecular density. A traditional detection technique, refractive index, provides direct detection of carbohydrate concentration with a nonspecific response. Coupling several of these detection techniques to measure the carbohydrate eluting from HPSEC allows the determination of molecular size, branching, conformation, and structure from the same sample. Thus, more information may be gained about starch structure, function, and their relationships.

Although most of these unique starches described have not been produced and used commercially, Cerestar USA, Inc., National Starch and Chemical Corporation, and Du Pont Agricultural Products Co. recently have applied for U.S. patents on starches from a number of double-mutant combinations. These starches and their patents will be discussed later.

V. ORGANIZATION OF CORN STARCH GRANULES

A. Granular Structure

The amylose, amylopectin, and intermediate material fractions of starch described in the previous section are tightly packed together to form tiny insoluble granules that are biosynthesized within the plant cell. The starch granules are formed inside the cellular organelle called the amyloplast, but the number of starch granules differs among cell types, depending upon the cell function. Although the granules seem to be distinct packets of material, there is no evidence of a membrane surrounding them. Normal corn-starch granules range in size from 5 to 25 µm in diameter,[1] depending on factors such as genetic background and growing conditions.[104] Wang et al.[33] determined that normal (Oh43) corn starch granules ranged from 6 to 17 µm in diameter, with an average diameter of 11.6 µm. Some researchers reported that *wx* corn starch granules are slightly larger than those found in normal corn.[104] Wang et al.,[33] however, noted a slightly smaller granule size for *wx* starch granules. The average granule size probably varies with the cultivar and environmental conditions, and likely with the method of analysis. The *ae* granules are generally agreed to be smaller than normal corn starch granules[33, 104] Table 2.3 shows the size distribution of starch granules from 17 corn genotypes all in an Oh43 inbred background. In other work, the diameter of the *Ae1*-5180 dominant mutant starch granules, with one to three doses of *Ae*, ranged from 4 to 18 µm in diameter.[77]

Scientists are still learning how the amylose and amylopectin molecules are arranged relative to each other, but the molecules seem to be distributed uniformly throughout the granule. The amylopectin has a crystalline cluster-type structure, whereas the amylose may be located in both crystalline and amorphous regions. A model proposed by Nikuni[105] showed amylose being concentrated in the amorphous region between, and separated from, the amylopectin molecules. But recent studies by Jane et al.[106]and Kasemsuwan and Jane[107] suggested that the amylose is interspersed among the amylopectin molecules and located in both crystalline and amorphous regions. Gallant et al.[108] proposed that the amylopectin lamellae are organized into spherical “blocklets,” which range in diameter from 20 to 500 nm, depending on the starch botanical source and location in the granule. They suggested that short, radial channels of amorphous material occur within the granules.

Starch granules generally can be grouped by morphology into four categories: generally spherical, generally angular, dimpled, and irregular.[109] The shape of starch granules in normal corn tends to be round, but the granules close to the germ acquire a polygonal shape as they become crowded during growth films. Granules from *ae* corn range from spherical to lobed to “snakelike” or elongated with the deformed granules becoming more abundant as amylose content increases. Figures 2.1A through 2.1C present scanning electron micrographs (SEMs) of granules from normal,

TABLE 2.3
Size of Starch Granules from 17 Corn Genotypes in an OH43 Background[a]

Source of Starch[a]	Average ± SD[b] (μm)
Normal	11.6 ± 4.5
ae	7.0 ± 2.1
du	7.8 ± 2.1
su	5.4 ± 2.6
wx	10.3 ± 2.6
bt1	6.1 ± 1.3
bt2	10.8 ± 3.4
h	13.8 ± 3.6
sh2	6.3 ± 1.9
ae bt1	5.6 ± 1.6
ae du	7.4 ± 2.3
du1 su1	6.9 ± 2.6
h sh2	11.2 ± 3.8
h wx	11.0 ± 4.4
sh2 bt1	5.4 ± 1.6
sh2 wx	10.2 ± 3.7
wx du	10.2 ± 3.6

[a] *ae* = amylose extender, *du* = dull, *su* = sugary, *wx* = waxy, *bt* = brittle, *h* = horny, *sh* = shrunken

[b] Average ± standard deviation of 30 starch granules, 15 each from two scanning electron micrographs

Source: Wang, Y.-J. et al., in *Cereal Chem.*, 70, 171, 1993. With permission.

wx, and high-amylose corn demonstrating these characteristics. Original SEMs of starch granules from combinations of single, double, and triple corn mutants, shown in two papers,[33, 104] demonstrate the impact of interacting recessive corn genes on granule structure.

B. Measurement of Starch Granule Organization

Native starch granules have a number of organizational structures allowing observation by several instrumental techniques. A characteristic birefringence appears when the granule is viewed under polarized light; the starch appears as a black Maltese cross with its center at the hilum (Figure 2.2). This overall spherulitic organization is influenced by starch molecules that radiate out from the hilum toward the periphery. A current theory is that the birefringence of granules is due to the orientation of molecular chains in the amorphous regions as well as to crystallinity of the molecules *per se*.[110] Consider, however, that stretched amylose or ball-milled granules may be very birefringent but not crystalline, and that high-amylose starch granules are not always very birefringent even though they are partly crystalline. The theory also is in contrast to earlier work suggesting that birefringence was caused by the crystallinity of amylopectin molecules.[111] The reasoning was that the intensity of the Maltese cross decreases in high-amylose corn starch, yet *wx* corn starch, which is highly crystalline, exhibits the same birefringence properties as does normal starch.

A different level of starch granule organization is seen in the polymorphic forms of starches in their native state, which can be studied by electron microscopy and X-ray diffraction. The spacings between crystalline and amorphous regions can be detected by observing electron-dense areas. Native starches exist predominantly in two polymorphic forms: the A starch, which is more

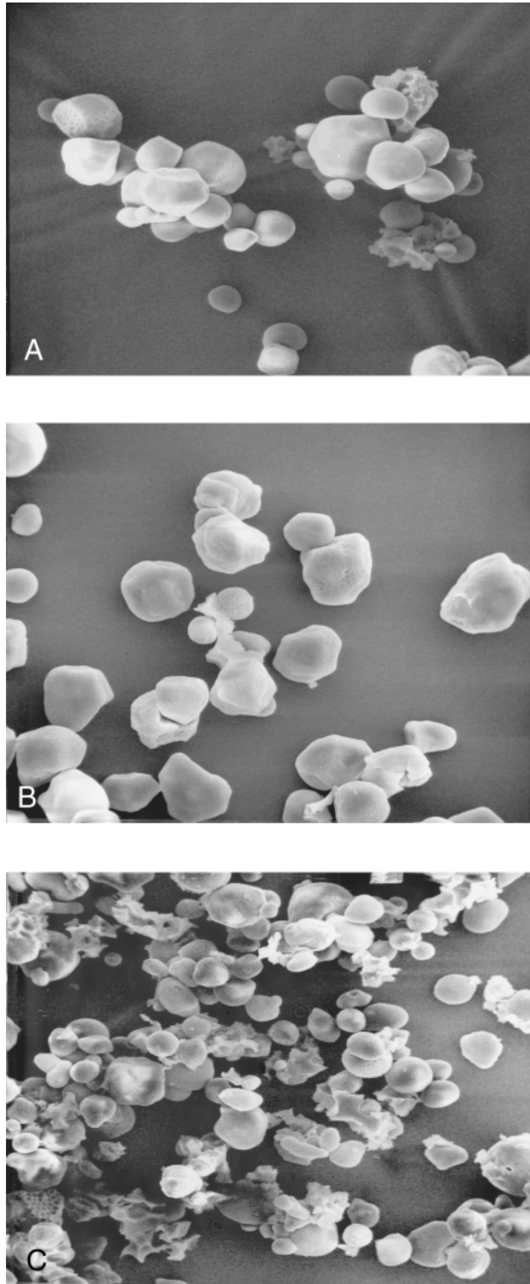


FIGURE 2.1 Scanning electron micrographs (magnification × 1000). (A) Normal corn starch from Oh43 inbred. (B) *wx* corn starch from *wx* in an Oh43 background. (C) *ae* corn starch from *ae* in an Oh43 background.

crystalline and characteristic of cereals and grains, and the B starch, which is less crystalline and characteristic of tubers and roots.[112] Starch from some plants (sweet potato, banana, horse chestnut, and tapioca) has a rare C-type structure in which there is no trend to crystallinity.[110] Both normal corn and *wx* corn have A starch, whereas high-amylose corn has B starch.[77,110] Differences in X-ray diffraction patterns of A- and B-type starches may be because A-type starches have amylopectin branch points present in both amorphous and crystalline regions of the granule, whereas B-type starches have most branch points clustered in the amorphous region.[93] C-type starches have a closely

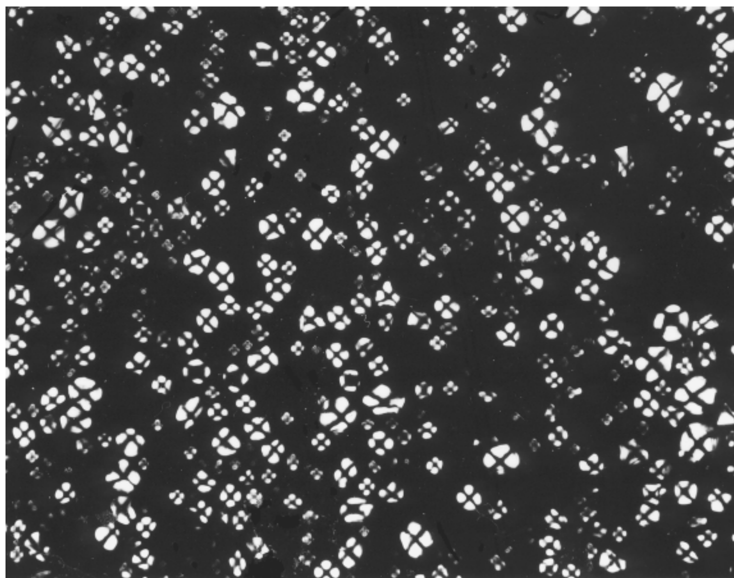


FIGURE 2.2 Photomicrograph of normal corn-starch granules under polarized light. (Magnification × 800.) The Maltese cross-pattern is typical of spherocrystals.

clustered branching pattern. These crystalline patterns fit nicely with the cluster-type models proposed for amylopectin that were discussed in Section IV of this chapter, or with the newer model proposed by Gallant et al.[108]

The presence of phosphorus in starches affects starch behavior. The phosphorus may be present as starch phophate monoesters, where it is bound directly to the starch molecules, especially amylopectin, or as phospholipids, where it is complexed within the helical chains of amylose and of long-branch chains of amylopectin. Normal corn starch contains essentially no phosphate monoesters, and, among cereal starches, the least amount of phospholipids (0.016% compared with approximately 0.05% for wheat, rice, oat, and millet[113]). In contrast, *wx* and *duwx* corn starches contain about 0.002% monophosphates. The *wx* starch contains no phospholipids, whereas *duwx* has a trace amount. The presence of phosphate-monoester derivatives in a starch increases paste clarity and paste viscosity, whereas phospholipids make starch pastes more opaque and decrease paste viscosity.[114,115] The importance of these and other functional properties is described in the following section.

VI. FUNCTIONAL PROPERTIES OF CORN STARCH

A. General Properties

1. Gelatinization

a. *The Process*

The starch granules present after isolation from the kernel are partly crystalline and, hence, water-insoluble. At room temperature, the granules can absorb about 30% of their weight in water through hydrogen bonding, a process that is reversible. Major irreversible changes in the physical properties of the starch do not take place until water and heat are applied simultaneously, a process referred to as "gelatinization." The heat creates kinetic energy within the starch granule, breaking existing hydrogen bonds and allowing penetration of the water into the granule. Amylose tends to leach out of the granule and, along with the amylopectin, becomes very hydrated, resulting in an increase in viscosity and clarity of the starch–water mixture as gelatinization continues. These changes also

cause a decrease in crystallinity, noted by the loss of birefringence under the microscope. The point at which birefringence first disappears defines the actual "gelatinization point." In an effort to standardize the terminology associated with basic starch phenomena, gelatinization was described as follows: "Gelatinization is the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization."[116]

The gelatinization process generally occurs in a narrow temperature range, with larger granules gelatinizing first, followed by the smaller granules. For corn starch in an excess of water (about one and one half to two parts water or more to one part starch), that range is from 61 to 72°C. Drier conditions increase the temperature of gelatinization and can result in incomplete gelatinization of the starch. A ratio of at least one part water to three parts starch is required for any gelatinization to take place.[117]

b. Methods for Measuring Gelatinization

Gelatinization can be studied by using physical, chemical, and biochemical methods.[118–120] These procedures provide an accurate means for examining the specific factors affecting starch gelatinization.

Light and electron microscopy — Microscopic examination of starch granules during gelatinization can be done by using a light microscope fitted with a hot stage. Swelling duration, degree of swelling, and swollen granule integrity and size can be determined. Observing loss of birefringence, as mentioned previously, requires an optical microscope with crossed polarizers and a heating stage.[118] Berry and White[121] describe a photometric device for recording the loss of birefringence. The scanning electron microscope (SEM) also has been used to study structural changes occurring during starch gelatinization.[122,123] The shapes of the granules can be observed during loss of birefringence.

Light transmission — Changes in light transmission of starches during various stages of gelatinization can be used to follow the process. A spectrophotometric method to measure the transmitted light was described by Beckford and Sandstedt,[124] and the instrument was modified by Longley and Miller.[125]

Viscometry — A common method for observing starch pasting behavior is by use of a Brabender viscoamylograph. A starch slurry of about 6 to 8% starch in water is heated in the instrument at a preset temperature and stirring speed for a specified length of time. As the granules swell with the application of heat and water, the viscosity of the mixture is measured and recorded in arbitrary units that reflect paste consistency. Differences in viscoamylograph properties among starch types and in starch slurries with added ingredients can be readily noted. The viscoamylograph is the preferred method for evaluating pasting, a process sometimes considered to be synonymous with gelatinization,[116] but a newer instrument, called a Rapid Visco Analyser (RVA), is preferred by researchers who have limited quantities of material for starch analyses.[126,127] The RVA is capable of simulating the values determined from the viscoamylograph, using only a fraction of the starch required for the latter analysis.

Swelling and solubility determinations — The swelling power of a starch can be measured by determining the weight of the swollen granules and their occluded water at various temperatures.[128] For example, at 90°C, the swelling power of potato starch is 350, compared with 65 for tapioca, 60 for waxy corn, and 16 for regular corn starch.

X-ray diffraction — The crystallinity of starch granules, indicating differences in organization or structure of the starch molecules, can be measured by X-ray diffraction. The method has been used to measure extent of gelatinization[129–131] and to determine relative amounts of crystalline and amorphous phases within the granule.

Differential scanning calorimetry — Differential scanning calorimetry (DCS) is perhaps the newest method adapted for use in studying starch gelatinization properties. Since

Stevens and Elton[132] first reported the procedure in 1971, DSC has been used extensively, with many reports appearing around 1980.[133–136] The application of DSC to starch gelatinization has considerably improved the understanding of this property.[137] Determination of starch gelatinization by DSC requires sealing a small amount of starch (1 to 4 mg, dry-weight basis) and the chosen amount of water in aluminum or stainless steel pans. The starch and water in the filled pan and a pan containing a reference material are then heated together at a specified rate in the differential scanning calorimeter. Differences in thermal properties between the sample and reference pans are recorded as the starch gelatinization properties. Parameters including onset, peak, and conclusion temperatures; temperature range; and enthalpies of gelatinization and retrogradation can be precisely measured.

Other — The polarized light microscopy, viscoamylograph, light transmission, and swelling or solubility methods, although older, are still useful for evaluating gelatinization. The SEM, DSC, and X-ray diffraction procedures are newer analytical methods that are now widely accepted. Several other newer techniques worth mentioning include an enzymatic procedure involving incubation of starch preparations with glucoamylase,[138] a small-angle light-scattering system,[139] and nuclear magnetic resonance.[140] A recent survey of starch scientists[116] revealed that the preferred methods for evaluating gelatinization are DSC and polarized light microscopy.

c. Factors Affecting Gelatinization

General properties — A starch's gelatinization characteristics are important to most areas of starch utilization. Five parts of corn starch (by weight) can completely immobilize 95 parts of water, but there are many factors affecting this action and the resulting properties. Extremely swollen granules can rupture, collapse, and fragment, causing a drop in viscosity, especially if overstirring of the mixture occurs. Care must be taken to avoid overmanipulation of a greatly hydrated system.

When gelatinized, normal corn starch, like most cereal starches, generally thickens to form a gel-like structure upon cooling. Gelatinized starches from roots or tubers, such as potato and tapioca, become very viscous upon cooling but form a colloid (sol) with undefined edges, rather than an organized gel. Also, the root starches tend to maintain greater clarity after cooling than do the cereal starches. Starch from *wx* corn behaves more like root starch than cereal starch.

Solvents other than water (liquid ammonia, formamide, formic acid, chloroacetic acids, and dimethyl sulfoxide) also can cause starch to gelatinize with heat, but these interactions are not as common or as important as those of water with starch. In food systems, the presence of many other ingredients affects the characteristics of the starch-thickened mixture. Ingredients such as sugars, salts, fats and oils, proteins, and acids are commonly present.

Effects of sugars on gelatinization — Sugars tend to compete with starch for water, thus decreasing the amount of starch gelatinization that can occur. Large concentrations of sugar, such as occur in sweetened puddings and fruit sauces, decrease the rate of thickening, the energy of gelatinization, and the final gel strength, with disaccharides exerting more effect than monosaccharides.[141,142] Sugars also elevate the temperature at which starch granules begin to thicken a liquid and make swollen granules more resistant to mechanical rupture after gelatinization.

Effects of lipids on gelatinization — Fats and oils in the form of triacylglycerols can decrease the temperature of maximum viscosity, allowing a corn-starch–water mixture containing 9 to 12% fat to gelatinize at 82°C rather than 92°C.[143] Of greater interest, however, are the effects of polar fatty components, such as monoglycerides and diglycerides, at concentrations of less than 1% (on a starch dry-weight basis); these can form complexes with the amylose portion of starch or with long outer branches of certain amylopectin molecules. Long starch-chains are hypothesized to form a helical structure around the fatty molecules.[110] The resulting inclusion complexes resist leaching from the granule and entry of water into the granule, altering the starch functional properties. Practical examples where these interactions are utilized include to following:

1. Addition of sulfonated oils to textile starch sizes as "softeners" and antiskinning agents.
2. Use of soaps to modify viscosity of starch sizings in paper coatings.
3. The former application of polyoxyethylene monostearate as a bread softener.
4. Use of monoglyceride to alter the paste consistency of dehydrated potato flakes.
5. Use of glycerol monostearate and other mono- and diglycerides to prevent "stickiness" in cooked rice kernels.[117]

Monoglycerides, diglycerides, and other surfactants have been used as bread softeners for years to reduce staling in bread as it ages. Surfactants containing fully saturated fatty acids of long chain length, such as stearate, provide the best antistaling action.[144,145] Polyoxyethylene monostearate and glycerol monostearate have been used successfully, but the former compound is more effective.[146]

Both time and temperature are necessary for interaction of the fatty adjunct with starch, so the two ingredients must be present together during gelatinization. In general, the long-chain fatty adjuncts that can complex with starch repress swelling, solubilization, skinning, bread staling, and stickiness of starches and cereal grains. In addition, they cause an increase in gelatinization temperature, an increase in the temperature of maximum viscosity, a decrease in the temperature of gel formation, and a decrease in gel strength.

Effects of salts on gelatinization — Low concentrations of salts generally found in food systems have little effect on corn-starch gelatinization or gel formation because of the neutrality of the starch. At experimental concentrations, however, where the starch to water ratio was 1:2 and sodium chloride (NaCl) concentration in the aqueous phase was included at up to 30%, large variations in wheat-starch gelatinization properties were noted.[142] Beginning temperature of gelatinization rose by 18°C with the addition of 9% NaCl and, with subsequent increments in salt level up to 30%, gradually fell by 9°C. The range of gelatinization was narrowed by a salt addition of 9%, but gradually widened as salt concentration was increased up to 30%. Similar effects would be expected with corn starch. Other salts besides NaCl may have similar or different effects on starch gelatinization, depending upon their nature as anions or cations.

Effects of pH on gelatinization — The pH of a system can have a great effect on starch viscosity. Acid reduces the thickness of a hot starch paste and firmness of the cooled paste because of acid hydrolysis of the starch molecules in the swollen granules. At pH values in the range of 4 to 7, normally found in most food systems, the acid concentration has little effect. At low pH, however, such as is found in some salad dressings and fruit desserts, extensive hydrolysis of the starch can occur, resulting in a dramatic decrease in viscosity, especially if heat and acid are present together. At a pH of about 10, the rate of starch swelling is greatly increased. This pH value is outside the range found in food systems, but the effects could be used in industrial processes.

Effects of proteins on gelatinization — Starch gelatinization and wheat gluten development provide the basic structure in most baked products. Although proteins and starches function together in these foods, their interaction is difficult to study because their basic macromolecules are very different.[147] It was thought that proteins might influence starch staling, but the primary effect of proteins in reducing the staling rate was dilution of the starch and not an interaction with protein.[148] Corn starch would be expected to behave in a fashion similar to that of wheat starch.

2. Pasting

The term "pasting" is sometimes used synonomously with that of "gelatinization," but the two terms have slightly different meanings. Pasting could be considered as the process by which a starch paste is formed, and this term is most often used in association with viscosity techniques.[116] Pasting is sometimes referred to as those changes occurring after starch gelatinization. The favored definition for "pasting" in the survey of starch scientists is as follows: "Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules."[116] This

definition distinguishes between the two terms by noting the sequential nature of the processes. During pasting of normal corn starch, amylose preferentially leaches out into the surrounding liquid; thus, a cooked hot starch paste consists of swollen granules suspended in hot water containing dispersed molecules of amylose. After cooling, the paste may have a firm gel-type consistency that holds the shape of a container (gel) or may simply be very viscous without sharp edges (sol), depending upon the nature of the starch. An instrument, such as the Stable Micro System TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY), can be used to measure the gel/sol strength and stickiness. Added ingredients also can alter cooled paste characteristics in ways similar to those described under the section on gelatinization.

3. Retrogradation

In a cooled starch paste made from normal corn starch, the amylose molecules tend to reassociate. The amylose molecules rebond to one another and to starch molecules on the outer edges of granules, forming a crystalline network. This recrystallization of gelatinized starch is known as "retrogradation." The term also has been used to describe events occurring during aging of a starch paste.[149] The most popular definition for retrogradation among starch scientists is as follows: "Starch retrogradation is a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure. In its initial phases, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears."[116] The DSC is preferred to other methods for measuring retrogradation, although light scattering and X-ray diffraction are also successful techniques.[116]

Not all cooked starch pastes undergo retrogradation to the same extent. Starches containing no amylose or amylose molecules that are too short have less tendency to recrystallize. Some chemically modified starches also are cross-linked with various chemicals such as phosphates to inhibit retrogradation. Normal native and high-amylose corn starches exhibit a strong tendency to retrograde. Foods such as gravies and sauces, thickened with normal corn starch, have poor freeze–thaw stability largely because of retrogradation of the amylose. The *wx* starches, and especially those that have been cross-linked, perform much better in frozen foods.

B. Properties of Native Corn Starch

Selection of a starch for a particular use requires consideration of the many starch properties discussed. The most important characteristics include temperature; range and enthalpy of gelatinization; tendency to retrograde; heat, acid, and storage stability; dispersability; cold-water swellability; nature of the thickened mixture (gel or sol); pasting characteristics; clarity of the thickened mixture; gel strength; and viscosity or extent of swelling.

In general, it is the ability of starch to form thick pastes that makes it a valuable ingredient in many foods. But native corn starch has limited usefulness in many commercial applications,[1] because it lacks free-flowing properties, or dispersibility of the granules, and cold-water solubility. There may be either excess or uncontrolled viscosity upon cooling and a cohesive or rubbery texture of the cooled paste. Starch also is susceptible to rupture and hydrolysis with extended cooking, manipulation, or exposure to low pH, and the gel lacks clarity and tends to retrograde after cooling.

Chemically modified starches were introduced to overcome these problems and to expand the usefulness of starch. The topic of modified starches, including their properties and uses, was thoroughly discussed by Wurzberg.[1] Modern efforts to modify the starch properties by natural means were begun with the development of waxy and high-amylose corn varieties. Starches from these corn mutants differ from normal corn starch in both structural and behavioral characteristics. In addition, recent efforts have been made to find native corn starches with altered structures that

may have desirable characteristics of their own or that may mimic those of modified starches. Properties of starches from corn populations other than normal Corn Belt dent are discussed next.

C. Properties of Starch from High-amylose Mutants

Commercial varieties of high-amylose corn produce starch containing about 65% amylose, although experimental lines can produce starch with up to 85% amylose or higher, as discussed previously. The amylose molecule is difficult to gelatinize, requiring temperatures greater than 100°C, and high-amylose starches exhibit this same property. Once cooked, the high-amylose starch paste is very viscous and forms a crystalline gel-like structure, having a strong tendency to retrograde. At 2% concentration, amylose can form a free-standing gel structure.[110] Recently, noodles made from a mixture of cross-linked tapioca starch and 17% high-*ae* corn starch were comparable to the clear noodles made from expensive mung bean starch.[150] Separated amylose can be formed into strong transparent films and fibers, resembling products made from cellulose, a natural plant fiber consisting of beta-*D*-(1-4)-linked glucopyranosyl units that is indigestable to humans. This "natural" food-fiber source, known as resistant starch, has captured the interest of nutritionists and plant researchers. Also, uses for starch amylose as a natural industrial polymer may increase as the demand for biodegradable products rises.

The association of amylose molecules with one another is generally extensive and involves chain segments of DPW of about 50.[151] Consequently, amylose gels are very rigid and do not readily melt. Ring et al.,[151] however, were able to demonstrate the presence of a melting transition at 153°C for a 12% potato amylose gel by using DSC.

D. Properties of Starch from *wx* Mutants

The *wx* corn starch, containing nearly 100% amylopectin molecules, produces thickened mixtures that are very different from those of the high-amylose starches. The *wx* starch is relatively easy to gelatinize and produces a clear viscous paste with a sticky or tacky surface, rather than one with sharp edges. This paste resembles pastes of root or tuber starches, such as potato or tapioca.

With no amylose molecules present, *wx* starch has less tendency to retrograde than do high-amylose or even normal corn starches. It was originally considered that only amylose was involved in retrogradation, but recent work has shown amylopectin involvement in the process. Researchers now know that the initial development of firmness during gelation of a starch paste is caused by the formation of an amylose matrix gel. The amylose is thought to fully retrograde by the time the paste has cooled to room temperature. The slow increase in firmness of a starch paste during storage for several days is likely because of the crystallization of amylopectin within the gelatinized starch granule.[151,152]

The interchain association for amylopectin involves linear outer chains with segment lengths of DPW of about 15, rather than 50 as mentioned for amylose. Thus, the amylopectin crystallization is weaker than that of amylose, demonstrated by the thermoreversible behavior (melting) of amylopectin crystals at 100°C when measured by DSC.[151]

Although amylopectin does indeed participate in retrogradation its pastes are still more stable than those of amylose. A paste made with native *wx* starch, however, is very sticky and even stringy, so *wx* starch is generally modified before commercial use.

E. Properties of Starch from Other Corn Mutants and Inbred Lines

Chemical modification techniques applied to normal, high-amylose, or *wx* starches (corn and others) have provided the industry with a huge array of available starches. Recent consumer interest in all-natural starches and more restrictive rules for regulatory approval of chemical processes have generated an interest in finding native natural materials to partly replace the chemically modified ones. For corn starches, natural variations could be created by various combinations of available

mutants and inbred lines. The structural characteristics of many of these potentially unusual starches were discussed earlier in this chapter.

The relation between the major structural features in starches (amylose vs. amylopectin) and function are fairly well understood, but the effects of the fine structural characteristics on function are far more difficult to decipher. The application of DSC to starch gelatinization, along with improved GPC techniques to study starch fine structure, has aided in this pursuit. Inouchi et al.[153] examined the DSC properties of starches from several corn genotypes (normal, *ae*, *wx*, *du*, and *su2*) at different stages of growth, demonstrating values for enthalpy of gelatinization declining in the order of *wx*, normal, *du*, and *su2* starches. The enthalpy for *ae* was undefined by DCS. Brockett et al.[154] found similar results, with enthalpy values of starches from the mutant combinations with sweet corn inbred Ia5125 declining in the order of *aewx*, *wx*, *ae*, normal, and *du*.

In a study in our laboratory,[155] starches isolated from the corn inbred line, Oh43, its single mutants [*ae*, brittle-1 (*bt1*), brittle-2 (*bt2*), *du1*, *fl2*, horny (*h*), shrunken-2 (*sh2*), *su1*, and *wx*], and the double-mutant combinations within Oh43, were analyzed for DSC and gel-strength properties. Many significant differences were noted among the starch types. For gelatinization, the starches of *wxdu1* and *sh2du1* had the highest peak-onset temperatures and *hwx* had the greatest enthalpy. Double mutants *aebt2* and *aedu1* had the highest peak-onset temperature for retrogradation. The gelatinization enthalpy peak for *bt1* starch had a characteristic low temperature shoulder and wide range. Most double-mutant combinations had higher peak-onset temperatures and greater enthalpies than did those of the respective single mutants for gelatinization and had lower peak-onset temperatures for retrogradation. For gel strength, the *du1* starch had the smallest values for firmness and stickiness among all samples. Double mutants generally had lesser gel-strength measurements than did those of single mutants *bt1*, *bt2*, *fl2*, *h*, and *sh2*, but greater than those of *du1*. Starches from these same single and double mutants in an Oh43 background were evaluated for their physicochemical properties,[156] and these data related to previously determined structural properties.[33] Amylose content determined from gel permeation chromatography was the most important structural characteristic affecting the physicochemical properties of starch. Amylose content was significantly ($p < 0.01$) correlated with blue value (r = 0.96) and lambda max (r = 0.81) and was negatively correlated with limiting viscosity number (r = –0.83), percentage of transmittance (r = –0.88), swelling power (r = –0.86), and peak viscosity on the viscoamylograph (r = –0.97).

Krueger et al.[157] examined the effect of inbred-line differences on the thermal properties of normal corn starch. Starches from different corn varieties had significant variations in thermal properties, especially in enthalpy. White et al.[158] demonstrated variability in thermal behavior among five open-pollinated populations of genetically variable corn. That study also showed significant differences among plants within the same population, indicating that genetic variability for thermal behavior of the starches and likely for starch structure may exist within populations. Pollak and White[159] noted sufficient variability in thermal properties of Corn Belt germplasm to conduct effective breeding for unusual functional traits. Li et al.[160] further demonstrated wide genetic variability in the thermal properties, as measured by DSC, of corn starches from 35 tropical corn populations representing a range of sources and maturity groups.

Structural characteristics and functional properties of starches from kernels of a *su2* dosage series were examined to determine whether the normal allele (*Su2*) was completely dominant to the recessive allele (*su2*).[22] Differential scanning calorimetry (DSC) revealed intermediate values for gelatinization onset and peak temperatures, range, total enthalpy, and retrogradation among genotypes possessing one and two mutant *su2* alleles. No effect of gene dosage on apparent amylose content was observed, thus confirming earlier findings; however, viscosity of the starch paste and gel strength of the starches and stability to retrogradation resulting from two doses of the *su2* allele exceeded those of both mutant and normal genotypes. Possibly, novel starch types can be achieved through development of dosage intermediate genotypes.

Sanders et al.[161] did DSC analyses on starches isolated from four mutant genotypes (*wx*, *aewx*, *duwx*, and *aeduwx*) from four inbred lines (Ia5125, I1453, S3-61, and W64A). All starches contained

no amylose. Within a line, the *aewx* genotype always had the highest peak temperature, but the actual value and the degree of difference varied with the line. Enthalpy was greatest for the *aewx* starches in the S3-61 and W64A lines. They also examined the fine structures of those corn samples from the group containing the *wx* gene by using size exclusion high-performance liquid chromatography (HPLC). Within each line, the *aewx* starches having the greatest amount of high-MW components had the highest peak temperature by DSC. The two samples with the shortest retention time (greatest MW) for the primary low MW peak had the two greatest enthalpies. The authors suggested that a greater proportion of B2 and B3 chains to B1 and A chains may be responsible for a higher peak temperature, and that the proportion of B1 to A chains is a strong determinant of enthalpy. Hizukuri[55] proposed a model in which the low MW peak would be composed of A chains (slightly shorter) and B1 chains (slightly longer), whereas the high MW peak would by composed of B2, B3, and longer chains.

For the *aewx* genotype, the line (Ia5125) with the greatest proportion of low MW amylopectin had the lowest enthalpy. In general, Sanders et al.[161] found positive correlations between the percentage of total area in the high MW peak and all DSC parameters and a negative correlation between the retention time of the low-MW peak and the DSC parameters. They concluded that the thermal behavior of the starches might be related to two aspects of the amylopectin structure: more chains of greater MW and a slightly longer population of lesser MW chains.

Other work in our laboratory has shown an environmental effect on DSC properties of starches from exotic corn populations grown in two environments.[162] The peak-onset temperatures for gelatinization and retrogradation were higher and ranges of gelatinization were less in starches grown in a tropical (Puerto Rico) rather than a temperate environment (Ames, IA). On the other hand, few differences in thermal properties were noted among starches from Corn Belt dent corn.[163]

These studies have only begun to unravel the links between starch structure and function. Much more research is needed to fully understand these relations and the influence of genetic background and environmental effects on starch structure and behavior.

F. Specialty Starches for Commercial Use

Some commercial applications, however, have resulted from studies on the functional properties of double mutants of corn. Cerestar USA, Inc., Du Pont Agricultural Products Co., and National Starch and Chemical Corp. have applied for U.S. patents on several of these starches. Patents by Wurzburg and Fergason[164] in 1984 and by Zallie et al.[165] in 1986 are for use of starch from the *wxsu2* corn genotype as a thickener with improved low-temperature stability in the first instance and as an antistalent in bread in the second instance. Wurzburg and Fergason[164] claimed that a sol made with the *wxsu2* starch could withstand at least one freeze-thaw cycle more than a sol of a native *wx* starch. Zallie et al.[165] found bread containing the *wxsu2* starch or flour to have a softer, moister crumb after baking and a fresher texture and appearance after storage than bread made without it.

Several patents from Cerestar USA, Inc. are based on new starches that can replace chemically modified ones. Friedman et al.[166] claimed that sols made with starch from the *wxsh1* genotype had freeze–thaw stabilities superior to those made from chemically modified starches, and Friedman et al.[167] found thin–thick properties from starch of the *duh* genotype similar to those from chemically modified starches. The term "thin–thick" refers to the ability of a starch to maintain a desirable thin consistency during heat processing, but to then thicken appropriately during cooling. The characteristic is needed in canning of some food products. Thin–thick capabilities also are claimed in two other patents for mutant starches (*aedu*[168] and *dusu2*[169]). In addition, both starches have a high amylose content but gelatinize at temperatures much lower than *ae* starches, yielding energy cost savings to the user. Another mutant high-amylose starch (*aesu2*) has similar characteristics.[170] These new high-amylose starches are especially useful in foods, paper manufacture, and fiberglass sizing.

Starches described in three more patents (*wxfl1*,[171] *duwx*,[172] and *wxsh2*[173]) are said to exhibit properties similar to those of chemically modified starches, properties presumably including viscosity, clarity, and paste appearance. Starch from the *su2* genotype in an Oh43 background is particularly stable in acidic environments.[174] Reseachers from Du Pont Agricultural Products Co. obtained world[175] and U.S. patents[176] for the discovery of the grain and use of the starch from a triple mutant corn genotype, *duwxae*. The corn is heterozygous for *du* and homozygous recessive for *wx* and *ae*. Valuable properties for this new starch include higher paste viscosity, greater shear resistance, and greater acid resistance than normal and other mutant starches. In addition, it has a creamy texture when cooked, making it a suitable fat substitute. Another U.S. patent was issued for the grain with heterozygous *ae*, homozygous *wx*, and heterozygous doses of either *du, su,* or *sh*,[177] whose starch has a lower final viscosity than the *duwxae* but a decreased problem with retrogradation.

Researchers from National Starch and Chemical Corp. patented "low-amylopectin starch," a starch containing less than 10% amylopectin, and preferrably less than 5% amylopectin.[178] Further characterization of the starch identified the presence of 80% amylose, 5% amylopectin, and 15% low-MW amylose (intermediate materials). Gels made from the low-amylopectin starch did not exhibit syneresis during refrigeration for 5 months, so were exceptionally stable to retrogradation. The new starch also provided a very rigid gel with a quick onset of gelatinization and wide tolerance to a range of cooking temperatures. These properties were more pronounced than in gels made from the Hylon V (50% amylose) and Hylon VII (70% amylose) starches.[179]

The trend toward specialty starches for specialty uses is likely to continue as additional unique starches are found and developed into commercial products. New grains created by transgenic modifications and/or by traditional plant breeding will provide more and different starches, which can be used to study structure/function relationships, making additional searches much easier.

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3 High Amylose and Waxy Corns

Virgil Fergason

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I. INTRODUCTION

Ever since the discovery of its existence in North America, corn (*Zea mays* L.) has had a prominent role in the development of American agriculture. Somewhere in its history lies the secret for its extreme genetic diversity. This variability has made it possible over the past several decades to develop a multitude of unique varieties and hybrids, each of which has its own distinctive chemical and physical properties. Perhaps the most important factor for its continued persistence in our society is the capacity for corn to produce large quantities of desirable carbohydrates over a wide geographical area and its adaptation to an array of soil types, climatic conditions, and cultural practices.

II. STARCH: ITS ISOLATION, SEPARATION, AND IDENTIFICATION

Starch is the major storage carbohydrate within the plant kingdom and is found in all organs of most plant species. In excess of 95% of the commercial starch produced in the U.S. is from corn.[1] The corn endosperm consisting of approximately 87% starch is the major starch storage component of the corn kernel, and it comprises nearly 82% of the total grain weight.[2] Simple as it may seem, starch is actually a complex arrangement of carbohydrate components arranged in a variety of forms within individual starch granules.[1,3] Starch has been defined as a polymeric carbohydrate consisting of anhydroglucose units linked together primarily through α-*D*-(1→4) glucosidic bonds. Normal dent or the commonly grown corn in the U.S. possesses two types of polymers — amylose and amylopectin (Figures 3.1 and 3.2). They are present in corn endosperm in an approximate ratio of three parts amylopectin to one part amylose. Amylose may contain from 200 to 2000 or more anhydroglucose units linked together in a linear order by α-*D*-(1→4) glucosidic bonds. Amylopectin is a higher molecular weight polymer

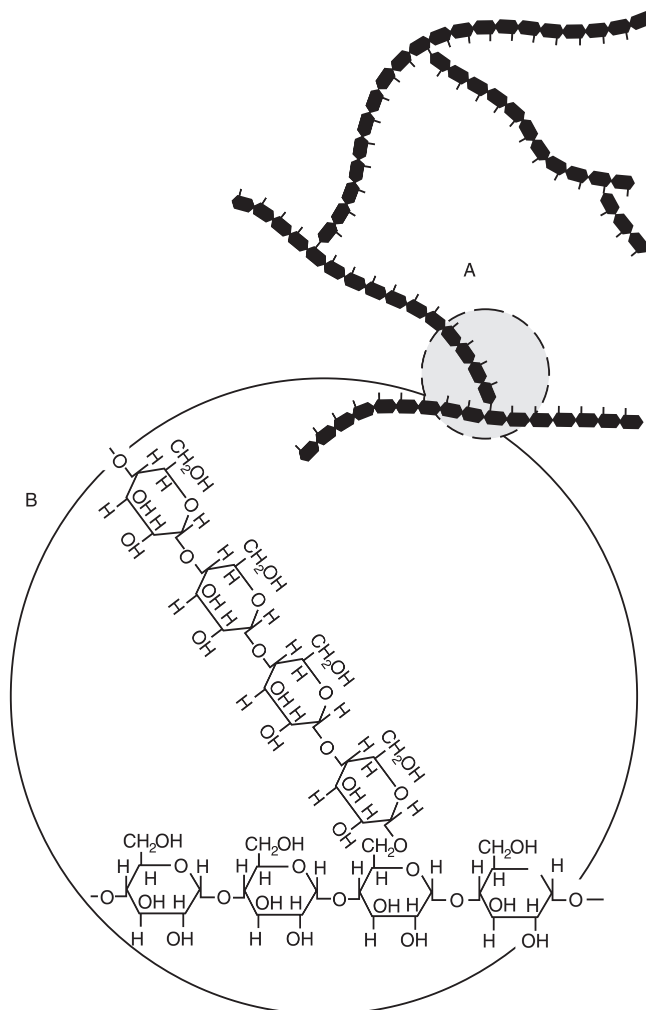


FIGURE 3.1 (A) Diagram of a fragment of a branched-chain molecule of the amylopectin fraction of starch. (B) Enlarged view of shaded section of diagram showing chemical structure and linkages at point of branching. (Paper presented by F. R. Senti before the 23rd National Farm Chemurgic Conference, Chicago, April 23, 1958. With permission.)

having anhydroglucose units linked together as in amylose by the α-*D*-(1→4) bonds, plus periodic side branches of anhydroglucose units connected at the carbon 6 position by α-*D*-(1→6) glucosidic bonds. Additional research has shown other polymers with some side branching occurring by α-*D*-(1→3) glucosidic bonding.[4] Starch consists not only of the normal amylose and amylopectin fractions but also of intermediates between these two polymers. These fractions may be amylopectin-like structures with a reduced degree of branching, or there may be side branches having a longer linear chain of anhydroglucose units. In addition, there are amylose types having a small degree of shorter side branches. All of these ramifications of starch types add to the difficulty in understanding the starch granule, its chemical and physical architecture, and the synthesis and deposition of starch in various plant organs. To gain a better understanding of the starch complex, the starch component must be isolated from plant tissue without causing modification of its physical and chemical properties. Whistler and Daniel,[5] and Young,[3] presented reviews of "starch fractionation," a procedure for the separation of amylose and amylopectin types. Once isolated, there are reliable methods for identification of these two starch polymers which are distinct in their ability to form iodine-starch complexes when treated

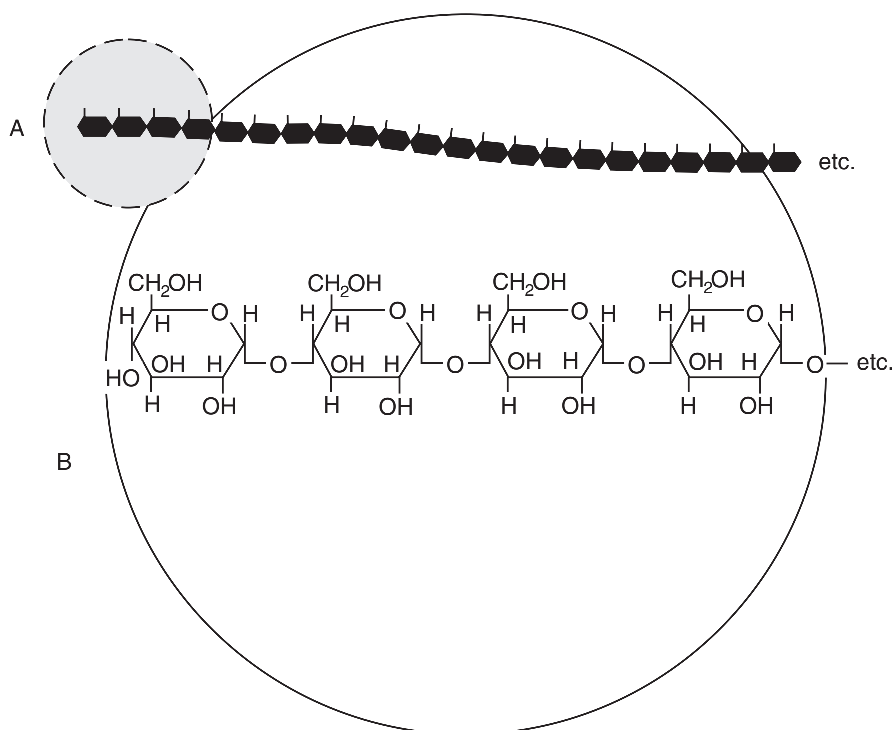


FIGURE 3.2 (A) Diagram of a fragment of a straight-chain or linear molecule of the amylose fraction of starch. (B) Enlarged view of shaded section of diagram showing chemical structure and linkages. (Paper presented by F. R. Senti before the 23rd National Farm Chemurgic Conference, Chicago, April 23, 1958. With permission.)

with a dilute iodine solution.[6,7] Amylose readily forms an insoluble complex with iodine, as opposed to amylopectin, which has a reduced affinity for iodine absorption; consequently, differential color strains are expressed. Iodine-stained amylose expresses a deep blue color while an amylopectin iodine complex results in a reddish-brown color. These colors are not absolute because the starch molecules themselves may be nonhomogeneous, thereby leading to differential staining. Length and number of side branches or length of the linear fraction affect the iodine starch complex and iodine absorbency thus resulting in different color reactions. No attempt will be made in this chapter to elucidate on starch fractionization procedures or the impact they have had on starch utilization. The progress made today in the starch industry would not have been possible without the wealth of information gained from the study of these two starch components whose isolation, separation, and identification were essential.

It seems appropriate at this time to pose the question: "Why is it necessary to determine the amylose-amylopectin ratio of plant starches and the unique and distinct properties of the starch complex in various plant species and in corn in particular?" Since the beginning of the starch industry, thousands of uses for corn starch have been discovered. These uses require specific starches or combinations of modified starch types. It is therefore imperative that the raw product be made available in the quantity and quality desired. Without adequate procedures for isolation, separation, and identifying these starch components, utilization objectives could not be achieved. Research involving a wide array of corn germplasm has led to the discovery of an enormous degree of variation in their carbohydrate complex. For example, there are corn germplasm sources available that range from less than 20 to 100% complement of amylopectin.

Various methods have been used to determine amylose-amylopectin ratios.[7–17] Potentiometric iodine titrations and spectrophometeric analyses have been used extensively to determine amylose-amylopectin ratios of various starches. The latter method is based on the absorbency of the starch-

iodine complex (also called blue-value procedure) and on relating the degree of absorbency to known amylose and amylopectin standards. The potentiometric method is based on the amount of iodine bound by 100 mg of starch and compared to the amount bound by a purified amylose standards. A review of these methods by Shannon and Garwood[7] points out that the amylose estimated by these procedures should be considered "apparent amylose" because the occurrence of branched chain components with long external chains can result in an overestimation of amylose. They also suggested that the presence of short-chain-length amylose components would result in an underestimation of the amylose component. Other investigations have shown similar variations in the architecture of the starch granules.[18–20] From the voluminous amount of research data available, it can be concluded that cornstarches cannot be precisely divided into amylose and amylopectin fractions, but they all blend together through various intermediates of various molecules sizes and weights.

It is obvious that the biosynthesis of starch and its deposition in the corn kernel is very complex and that there is an enormous amount of data available on starch synthesis. For example, by 1963, Sandstedt[21] reported that one volume on carbohydrate chemistry listed more than a thousand references. There are recent reviews which include the latest theories on starch biosynthesis and the enzymes involved in amylose and amylopectin synthesis.[13,17,19,22–34]

It is not the intent of this author to elaborate on starch synthesis in this chapter but rather to present a brief historical background in the development of high amylose and waxy corn and their significance as specialty type corns of today. Specialty corn is a common but somewhat vague description of corn which could be more clearly identified based on its chemical and physical characteristics and by its distinctive nutritional or industrial properties. Several corn endosperm mutations have been identified that modify both the equality and quantity of carbohydrates in the corn kernel. Many of these mutations are included in other chapters in this publication; therefore, this chapter will be devoted to only the high amylose and waxy corns and the specific mutations responsible for these special types. Since high amylose and waxy specialties differ so dramatically in many properties and have varied and different feed, food, nutritional, and industrial applications, they will be discussed in separate sections.

III. HIGH AMYLOSE CORN

A. Introduction

The terms "amylose" and "amylopectin" were used about 50 years ago to describe the straight-chain and branched-chain components of starch.[35] Previously, very little was known about the structure or identity of these starch components. In 1942, Shock[36] reported on the isolation of pure amylose and amylopectin starches. Common commercial starches used until then were actually a mixture of amylose and amylopectin. Then in 1943, Bates et al.[9] reported on a new analytical method for estimating the amylose content of plant starches. The results from these early investigations kindled renewed research interest into the use of these unique starch components in the starch industry. Subsequent discoveries and advancements in starch fractionation procedures provided more incentives for expanding the use of these purified starch fractions in various food and industrial applications. However, the fractionation of normal starch fractions to obtain higher levels of amylose starch is very costly. Likewise, this method provides large quantities of amylopectin starch which can be obtained more economically from waxy corn, which is discussed later in this chapter.

Various projects were initiated by several research scientists in an attempt to find plant sources having naturally enriched levels of amylose starch production. Subsequently, amylose analyses were made on a large array of various cultivated plant species including peas, barley, wheat, rice, sorghum, potatoes, and corn.[3] Results from these investigations included reports of corn endosperm mutations having higher than normal amylose contents.[4,7,23,37] Dunn et al.[38] reported an amylose

content of 77% from a triple recessive isolation of *du su su2*; however, the total starch content of this triple recessive mutant combination was reduced substantially. In addition to these studies involving endosperm mutations, surveys were made among many domestic and foreign sources of corn germplasm to find higher levels of amylose synthesis. In 39 different Indian corns from North, Central, and South America, amylose values were found to range from 22.2 to 28.3% with averages at the normal level of nearly 27% amylose.[39] Deatherage et al.,[40] in a survey of 200 domestic inbreds and varieties including more than 75 foreign varieties, reported amylose values ranging from 0 to 36%. The first real breakthrough in obtaining higher amylose starch levels without corresponding significantly lower total starch production came in 1952 with the discovery of the *ae* gene located on Chromosome 5.[41] Other mutants at the *Ae* locus have subsequently been reported.[42] These alleles, when compared to the original *ae* mutant, were found to be associated with slight modifications in amylose-amylopectin ratios as well as some alleles having starches with small amounts of a short-chain type amylose.

Phenotypically, the presence of *ae* can be identified very easily in most germplasm sources by the expression of a tarnished endosperm characteristic.[43] In some genetic backgrounds there also may be varying levels of wrinkling of the pericarp. This wrinkling phenomenon is probably associated with a reduction of sugar conversion to starch, subsequently resulting in a partial collapse of the endosperm. Immediately following the discovery of this amylose-enriching recessive gene, breeding programs in both the private and public sectors were started with objectives to develop hybrids having commercially desirable agronomic qualities and high amylose starch production. "Amylomaize" was proposed as the name for this new high amylose specialty corn.

B. Breeding High Amylose Corn

Early in the history of amylomaize, essentially nothing was known pertaining to the inheritance of *ae* and its epistatic interaction with other starch regulating genes. Subsequent work with *ae* and its interaction with other endosperm starch mutations revealed extensive variations in the level of amylose synthesis.[23,41,43,45] Vineyard et al.[46] reported amylose contents ranging from 36.5 to 64.9% from crosses between 135 different inbreds and a common *ae* source. Independent researchers suggested that "modifier genes" from different germplasm were interacting with the *ae* gene to produce various levels of amylose.[43,46] Later work involving amylose synthesis associated with several mutant alleles at the *ae* locus found endosperm starch having from 56.6 to 64.5% amylose.[42] Those differences were attributable to the epistatic effect of the major *ae* allele and its modifiers, thus supporting the theory that a range in amylose values of this magnitude is attributable to the effects of an unknown number of modifiers, interacting with the recessive *ae* gene. Phenotypic expressions may vary among inbred lines converted to high amylose starch production.[41] Fullness of kernel, which is a result of reduced starch production, is the most obvious variant and may continue to be of greatest concern to the breeder because total starch deposition within the endosperm is a major component of grain yield. Early studies of amylomaize revealed that high amylose starch granules differ significantly from those in normal dent corn.[20,47,48] No theories have been proposed to this date to explain why the presence of irregular shaped starch granules increases as the level of amylose synthesis increases. Other studies have shown a decrease in typical angular granules of normal corn with a corresponding increase in very irregular sausage-shaped granules as amylose synthesis increases.[18,20,21,37,49–51]

The primary objective of any corn breeding program should be the development of agronomically superior hybrids with reliable high performance standards over a wide array of growing conditions. In the early 1950s, Bear Hybrid Corn Company, with support from the National Starch and Chemical Company, began an extensive breeding program to incorporate the *ae* gene into agronomically desirable inbred lines and broad-based germplasm sources. It is not possible to phenotypically determine differences in levels of amylose starch production within the homozygous *ae* background. Therefore, the success of a high amylose breeding program is partially dependent

upon chemical starch analyses to determine the relative levels of amylose starch syntheses within selected samples from various breeding populations. The Northern Utilization Research and Development Division Laboratory of the U.S. Department of Agriculture at Peoria, IL, performed this analytical service for selected private and public breeding programs until the early 1960s.

The accuracy of amylose determinations is of utmost importance because it is essential in amylose breeding programs to select for very small inherent differences in amylose values; therefore, the error in determination should not exceed the selection differential if breeding progress is to be expected. Consequently, specially designed studies within various amylose breeding programs have provided information which has added to the knowledge needed to enhance the effectiveness of amylose breeding. Consistent progress in increasing the inherent amylose content of corn is dependent upon the successful separation of variations in amylose synthesis induced by genetic vs. environmental influences. Differences in amylose content of varying magnitude have been associated with many factors which include genetic effects as well as environmental influences. Fergason et al.[52] were among the first to provide evidence of kernel position effects on amylose content between ear zones and to demonstrate the occurrence of unequal distribution of amylose within the corn endosperm. Amylose analyses of samples taken from separate ear zones of high amylose inbred lines revealed amylose contents of 71.9, 69.8, and 67.4% for the base, middle, and tip ear zones, respectively. Similar trends, although of a smaller magnitude, were indicated when amylose analyses were performed on samples taken from different ear zones of four high-amylose hybrids. Highly significant differences in amylose content between endosperm portions were also found. These data revealed average differences as great as 4.8% amylose among starch samples taken from the middle, tip, and crown endosperm portions. Even though this represents a limited number of samples from only a few germplasm sources, it does provide consistent evidence that proper sampling procedures and analytical techniques are very important in any amylose breeding program. These data reflect trends similar to those reported by Boyer et al.,[30] in which they found that an increased amylose percentage was correlated with the physiological age of cells and that larger granules contained a higher amylose percentage. They reported that the basal endosperm cells begin starch biosynthesis late in kernel development and contain small granules with low amylose values.

Considerable evidence exists that environmental effects and cultural conditions can affect the chemical composition of corn grain.[53–58] Initially, Fergason and Zuber[59] reported on the environmental effects on the amylose starch level of six inbred lines grown in eight different states for a period of three years. The six inbreds in their study included one normal dent, four inbred lines which were homozygous for the *ae* gene, and one inbred homozygous for the *dull* (*du*) gene. Small, but highly significant differences were found for both location and year effects, but the differences in amylose content associated with location effects were greater than the difference associated with year effects (5.4 vs. 1.8%). Amylose differences attributable to locations, years, and all interactions were statistically highly significant, thereby supporting the theory that high amylose starch synthesis is associated with complex genotypic-environmental interactions. Correlation coefficients of –0.81 between amylose and degree days, and between amylose and average temperature during the growing seasons suggest that temperatures may have a significant impact on amylose starch synthesis.[59]

Would similar results be expected from environmental effects upon high amylose hybrids? To answer this question, four high amylose single-cross hybrids were grown at 12 locations in 1960 and 1961.[60] Again, highly significant differences in amylose synthesis occurred among entries, locations, and years with the greatest average differences of 7.8% amylose attributable to location effects. Likewise, correlation coefficients of –0.73 between average amylose content vs. degree days for each of the 2 years indicated that cooler temperatures during the growing season resulted in the highest amylose content.

Among other external factors that could influence amylose synthesis is a reduction of photosynthetic leaf area of the plant resulting from hail, leaf diseases, and insect feeding. Loss of leaf area as a result of any of these conditions would have to be considered by the high amylose corn breeder in interpretation of results or before making selections to be included in subsequent breeding

populations. Results from a study on the effect of leaf removal on amylose contents of corn endosperm did reveal an association between a reduction in leaf area and lower amylose content of endosperm tissue.[61] The magnitude of these differences was greater among inbred lines compared with differences noted among hybrids (2.5 vs. 0.75%). As expected, the impact on reduced amylose deposition in the endosperm was more pronounced by removing six top leaves when compared with the removal of six bottom or six alternate leaves. Additional studies reported by Helm et al.,[62] which were designed to determine the effect of planting date on high amylose hybrid performance, revealed that delayed planting was associated with increased amylose levels and lower grain yields. Their data indicated an inverse relationship of amylose content and accumulative degree days. They concluded that late planting may be advantageous for maximum amylose content but only with a sacrifice of lower grain yields and reduced performance for other agronomic characteristics.

Nearly 10 years after the discovery of the *ae* gene, the first commercial production and subsequent wet milling of high amylose corn grain occurred. This grain contained an amylose content in the mid 50% range. However, there was immediate interest in obtaining corn grain having 70% or more amylose for use in the starch industry. Continued and increased emphasis has been placed on breeders to develop agronomically superior high amylose hybrids possessing even higher concentrations of amylose starch. Corn breeders have continued to search for new breeding techniques or tools to enhance the effectiveness of their high amylose breeding programs. Perhaps, differential levels of amylose concentrations within corn pollen could provide some assistance to the breeders for selection within their breeding populations. However, unlike the occurrence of low amylopectin starch content from waxy pollen, there is no correlation in amylose content between the pollen and endosperm of high amylose grain.[46,48,63]

The requirements in a high amylose breeding program are broader in scope than in most other programs. It is necessary to consider not only all the usual agronomic requirements of normal dent corn breeding programs but also the level of amylose desired for commercial utilization. The effectiveness of a high amylose breeding program, as in any other, is dependent upon the type and level of genetic variability present in the available breeding populations, and the ability of the breeder to select for the most desirable combinations of the genetic factors involved. Within an amylose breeding program, progress in the development of high amylose hybrids must also emphasize the incorporation of suitable genetic modifiers of the amylose extender gene. Based on prior knowledge of the genetics involved in high amylose starch synthesis, breeders can be confident that the differences between various levels of amylose in breeding selections homozygous for *ae* must be attributable to the accumulation of specific modifiers of *ae*. Presumably more modifiers are required to reach the 75% level of amylose synthesis than to obtain the 65% level or lower. Prior research pertaining to gene dosage effects at the *ae* locus on amylose content of corn endosperm indicated a partial dominance of the wild type *Ae*.[64] It was determined, however, that all genotypes must be homozygous at the *ae* locus so that selection for the accumulation of *ae* modifiers can be achieved.

Earlier research indicated that a significant amount of additive gene action was involved in the inheritance of amylose starch synthesis.[65] When the breeding of high amylose hybrids was still in its infancy, the belief existed among some breeders that the development of amylomaize hybrids would be a simple matter of backcrossing the amylose-producing genes into inbred lines and then combining these lines in various hybrid combinations.[45] They also emphasized that "not only does the recurrent parent background affect the amylose content, but it also affects the phenotypic expression of the kernels." Some dent inbreds that were converted to amylose types produced completely full kernels while others revealed varying levels of collapsing of the crown portions of the kernel, and some gave translucent kernel phenotypes as opposed to the more opaque normal types. In some germplasm sources there were varied frequencies of complete collapse of the endosperm. Presumably in these types, there is little or not starch conversion. This phenomenon increases the difficulty in choosing which normal dent inbreds to convert to high amylose production.

Breeding procedures have been proposed for the development of agronomically adapted amylomaize hybrids.[45,66] These procedures included an alternate backcrossing and selfing sequence for the development of high amylose inbred lines. A minimum of three cycles of backcrossing was proposed. This proposal has serious limitations, especially if the high amylose nonrecurrent parent has undesirable agronomic regulating genes linked to *ae*; therefore, several backcrosses might be necessary to break the linkages and, subsequently, select against these linkages. In addition, unless the recurrent parent possesses the necessary modifiers for the desired level of amylose syntheses, the backcross procedure may have limitations for achieving the objectives set forth. If only the nonrecurrent parent possesses the modifiers necessary to achieve a specific level of amylose synthesis, then the size of the breeding population becomes a very important limiting factor. Large populations would, therefore, be required to be more assured of obtaining the necessary combination of genes in the selfing generation before making the next backcross. However, if the modifiers are supplied primarily by the recurrent parent, then it becomes much easier to select for the desired level of amylose syntheses. There is no clear answer to the question of how many generations of backcrossing will be required to obtain converted high amylose inbreds. Pleiotropic responses will definitely affect this answer. Some inbreds can be more readily converted to higher levels of amylose production than others, and some inbreds are not conducive to the conversion program. However, the backcross procedure was relatively successful in some of the earlier high amylose breeding programs.

Other amylose breeding procedures have also been very successful in the development of high amylose inbreds and their subsequent hybrid combinations. The relative short duration of hybrid longevity in today's agriculture places added pressure on corn breeders to develop new and improved hybrids as fast as possible. A backcross procedure in a high amylose breeding program has limitations in meeting the demands for the rapid development time of new amylose hybrids. This implies that the use of backcross conversion of the best dent lines should not be the only breeding procedure of a high amylose breeding program. Therefore, alternative breeding methods have been used to enhance the effectiveness of these amylose programs.

Pedigree selection has become a very reliable breeding method in development of new amylose hybrids. As expected, difficulties in utilizing this method are compounded as the degree of dissimilarities between parent breeding populations are increased. Pedigree selection does, however, offer the breeder flexibility in choosing among several options for meeting breeding objectives. It is not the intent of the author of this chapter to elaborate on the actual procedures or the germplasm sources that have been most applicable to the development of inbreds presently being used in commercially available high amylose hybrids. It should be emphasized, however, that the number and size of kernel samples selected for amylose starch analysis within a breeding population are very important components of a high amylose breeding program, and can become a major cost factor in conducting an effective amylose development program.

Through the 1970s, only two types of high amylose hybrids were used in commercial production: Class V containing amylose percentages in the 50% range and Class VII which normally possesses from 70 to 80% amylose starch. Progress in amylose starch utilization has continued to generate interest for higher levels of amylose beyond the 70% range. Special breeding programs are necessary for the development of amylose inbreds having amylose values at the 70% level and above. Experience has shown that the most breeding sources do not possess the assortment of modifier genes needed to reach these high amylose percentages. Therefore, some cyclic breeding system is needed to accumulate and select the favorable alleles involved in amylose starch synthesis prior to employing a regular inbred development program. In a presentation at the 1958 National Chemurgic Conference in Chicago, a participant implied that it would not be possible for corn breeders to develop breeding populations having amylose levels above the 80% level.[67] He also indicated that it would be necessary to discover at least one new gene for regulating amylose synthesis before amylose contents could be raised to 90% or higher. However, by 1958, Zuber et al.[43] had already broken the 80% amylose barrier. This report gave others the encouragement that even higher levels of amylose synthesis were attainable.

Early in the amylose development work, a form of recurrent selection seemed to be an acceptable breeding method for increasing the amylose levels in breeding populations; subsequently, recurrent selection has become an integral part of the Custom Farm Seed Amylose Breeding Program. An example of the progress made after ten cycles of recurrent selection is shown in Figure 3.3. Additional selfing and selection within populations taken from this program resulted in breeding sources having in excess of 94% amylose starch. These selections were used as parents in additional breeding work for development of high amylose inbreds for use in Class VIII or Class IX hybrids. This type of program definitely requires a commitment to long-range breeding programs with a realization that very large sample numbers are required to have any reasonable assurance of success in developing Class VII, VIII, or IX amylose hybrids. To conduct a high amylose breeding program for the development of amylose inbreds in the Class V through Class IX category, it is reasonable to assume that several thousand starch analyses per breeding generation may be required. The larger numbers would be required for those programs having limited amylose converted germplasm from which to start a breeding program. In addition, the number of analyses required would be greater with breeding objectives designed for increasing the levels of amylose synthesis beyond the 70% level in various breeding populations.

In 1979, Custom Farm Seed, a division of National Starch and Chemical Company, purchased the specialty breeding program of Bear Hybrid Corn Company which included both the amylose and waxy germplasm breeding program. Since that time Custom Farm Seed has been the only seed company developing and marketing proprietary Class V, VI, and VII high amylose hybrids. These hybrids have been developed from germplasm utilizing the *ae* gene and associated modifiers.

In 1988, Stinard and Robertson,[68] described a dominant *amylose-extender* mutant (*Ae*-5180) that they discovered in a mutator population. They reported the phenotype expression of *Ae*-5180 in several germplasms as varying from “slightly shrunken and tarnished to wrinkled sugary to

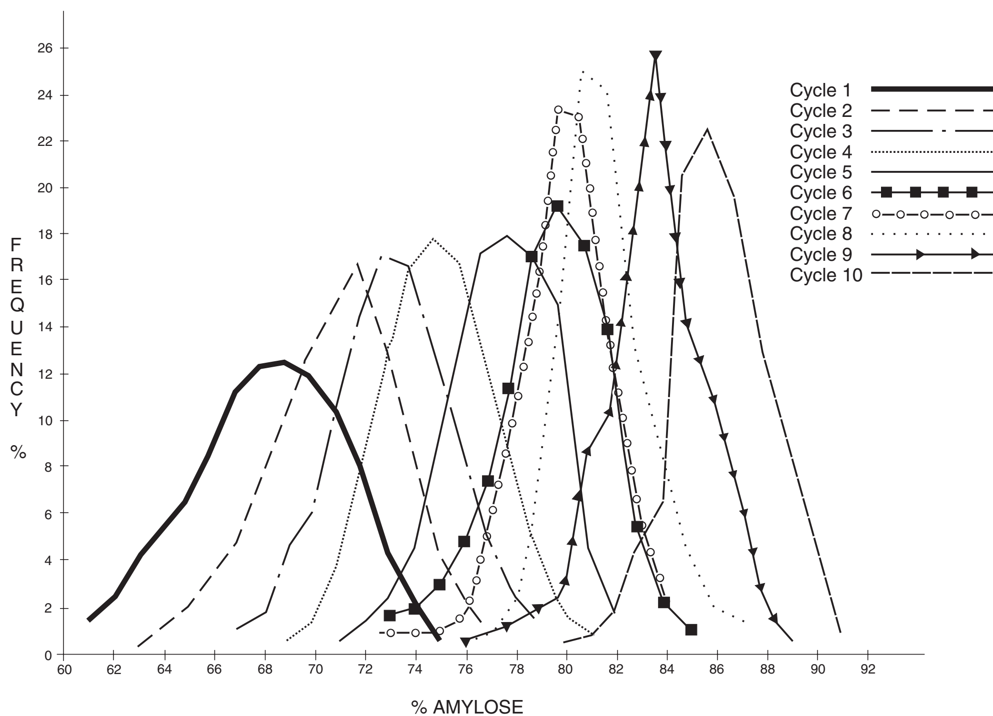


FIGURE 3.3 Progress from ten cycles of recurrent selection for increased amylose synthesis.

brittle" regardless of whether it is present in the endosperm as one, two, or three doses. This mutation was placed on chromosome 5 and was shown to be a dominant allele of *ae* (amylose-extender). This dominant allele is fully female-transmissible but may have a reduced transmission through the male. They also reported the presence of maternal effects (similar to those found with the recessive *ae*) when crossing *Ae*-5180 sources by various dent and flint inbreds. For example, the inbred B73, when used as a female in crosses involving the *Ae*-5180 genotype, results in a glassy, near brittle phenotypic expression. The use of the dominant amylose extender gene in high amylose hybrid production presents some potential problems for maintaining purity of commercial grain. Foundation and commercial seed impurities would be areas of concern to the seed producer and would be more difficult to determine compared with those foundation and commercial lots produced from the recessive (*ae*) germplasm. A series of questions are yet to be answered in reference to the A*e*-5180 allele, its implications in high-amylose breeding programs, and the subsequent use of high amylose hybrids from these development programs in the specialty starch industry. Perhaps history may eventually reveal that the discovery of the dominant amylose-extender allele did not have any significant impact on pre-existing amylose breeding programs.

C. Production and Management of Amylomaize

Amylomaize grain production comprises a very small fraction of the total corn acreage in the U.S. It is normally produced only by farmers having contractual agreements with domestic wet milling companies or with exporters of specialty corn grain. Only two corn wet milling companies in the U.S. currently contract for and process high amylose grain. The National Starch and Chemical Company, with wet milling facilities at Indianapolis, IN, and Kansas City, MO, is the largest consumer of this specialty. The only other remaining U.S. wet miller still involved in the use of high amylose grain is the American Maize Products Co. Essentially 100% of the high amylose seed currently being used in the U.S. has been developed, produced, and marketed by Custom Farm Seed of Momence, IL. Two classes of amylomaize grain are currently being utilized by the wet millers: Class V hybrids producing grain containing approximately 55 to 60% amylose starch and Class VII hybrids having a 70 to 80% amylose starch content.

Amylomaize hybrids require special management and cultural requirements to provide more assurance of optimum grain production of acceptable quality and purity. High amylose seed has slower germination and subsequent emergence rates than normal dent types. Consequently, recommendations are made to the grain producer that amylose should not be planted in cool wet soils. Other than some reductions in planting rate, and having adequate field isolation for maintaining grain purity, the cultural requirements for amylomaize are similar to those recommended for normal dent corn production. Grain yield potential of high amylose hybrids is less than most commercially grown dent or waxy hybrids. Test plot results from Custom Farm Seed involving 5 years of replicated yield trials revealed yield differentials of 20 to 25% (Table 3.1) when amylomaize hybrids were compared with dent and waxy hybrids. It is not unusual for some grain producers to compare their best dent or best waxy hybrid yields with that of the average yield performance of their amylose hybrids. Using this criterion for comparison, larger differential could occur. Realistically, however, performance should be based on all hybrids produced under comparable cultural and environmental conditions. Premiums are paid to the grain producer to compensate for yield differentials and special production, harvesting, and storage requirements.

Since high amylose starch synthesis is regulated primarily by a recessive gene (*ae*) and its modifiers, it is vital for the producer of amylomaize grain to adhere to specific production practices. Precautions should be taken to minimize the amount of foreign (non-amylomaize) pollen contamination within the high amylose production fields. Planting amylomaize hybrids either too close to non-amylomaize fields or by planting amylomaize in fields having the potential of non-amylomaize volunteer plants could result in foreign pollen contamination and reduction in desired grain purity. Preventive measures must also be considered at harvest, drying, shelling,

TABLE 3.1
Comparative Grain Yield Performance of Dent-Waxy with Amylose-Maize Hybrids

		Dent-waxy hybrids		High-amylose hybrids			
Number of locations[a]	Year	Average (q ha^{-1})	High (q ha^{-1})	Average (q ha^{-1})	High (q ha^{-1})	Average vs. average (%)	High vs. high (%)
3	1984	93	112	68	84	27	25
3	1985	95	114	75	90	21	21
2	1986	82	102	65	84	21	18
3	1987	96	116	79	92	18	21
2	1989	111	127	89	100	20	21
	Avg.	95	114	75	90	21	21

[a] emAll locations involve 48 hybrids in each category.

transportation, and storage to reduce the risk of non-amylomaize grain contamination and the subsequent rejection of putative amylomaize grain by the consumer or processor. Since the cost of amylose grain to the wet miller may be more than 50% higher than normal dent, it is imperative that the process not only receive desired purity, but also that the grain must possess the necessary physical and chemical qualities. Improper artificial drying of high amylose grain can reduce the value to the processor. Therefore, strict guidelines for proper drying procedures must be rigidly followed by the grain producer to prevent deterioration of the milling and starch qualities of the high amylose grain.

Experienced farmer producers of amylomaize grain have learned that this enterprise can generate additional profits beyond their normal dent corn production program. The key to this success is recognizing the best cultural practices required for optimum amylomaize grain production and subsequent strict adherence to them. Since amylomaize grain garners a higher premium to the producer, it behooves him to maximize his profits through good management.

D. Utilization of High Amylose Grain

Space allocation for this chapter does not permit a complete or even an adequate review of the complexity of uses for amylose starch in various industries. Fortunately, however, references are available which are excellent compendiums covering a multitude of applications for amylose starch.[3,69–77] Included in these references are examples for the use of this unique starch in the food, paper, textile, corrugating, and adhesive industries. Extensive applications for high amylose starch within each of these areas are expounded upon, thereby revealing the tremendous impact that amylose starch has had on the development of many consumer products. Young,[3] in an excellent review of the amylose starch, lists over 30 areas where amylose provides a significant function. This list is being expanded quite rapidly as new developments in amylose starch utilization are being discovered. As stated earlier in this chapter, the amylose component of starch possesses unique chemical and physical properties which result in specific functionalities. Usually some form of chemical or physical modification of the amylose starch molecule is required prior to its actual use in most industrial or food applications. These modifications enhance its functionality, thereby expanding its usefulness in various applications. For example, amylose starch when released into solution readily hydrogen bonds to form rigid opaque gels.[70] High amylose starch gels rapidly with subsequent formation of high strength gels which are useful in the confectionery industry because some types of candy require a stabilizer to give shape and integrity to these candy pieces. By using amylose starch in making candy gum drops, the molding and curing time has been reduced at least sixfold or more, thereby resulting in lowering production costs as well as increasing the production capacity of the candy manufacturer.

Amylose starch serves other functions in the food industry, including its use as a thickener in various puddings and processed foods.[3] It can be used as a binding agent for preparing dehydrated potatoes or as a coating to reduce oil absorption of deep fat fried potatoes. Tomato paste and applesauce texture is enhanced by the addition of modified amylose starch.

Amylose starches are used extensively in the corrugating and adhesive industries as a carrier because of their resistance to shearing stresses, high film strength, and water resistance. Just a few years after the first commercial production of high amylose hybrids, amylose starch was reported as having film-forming properties similar to cellophase.[78,79] Subsequent research eventually led to the use of amylose starch in many types of films and packaging coatings.[3] Amylose films are characterized as having excellent transparency, flexibility, tensile strength, and water resistance.

Many other uses of amylose starch in various industrial and food applications are known, but perhaps the greatest break-through in the use of amylose starch has occurred recently.[80] An exhibit by the National Starch and Chemical Company at the 1990 Chicago Packaging Expo introduced a unique packing material made from Hylon VII® cornstarch. This loose fill packing referred to as ECO-FOAM™ visually resembles the polystyrene foam "plastic peanuts" widely used in the packaging industry. Polystyrene foams, however, have drawn criticism as a potential landfill problem due to both their bulky nature and bio-degradability resistance. ECO-FOAM is described by the National Starch and Chemical Company as environmentally friendly as it is composed of 95% amylose starch which is almost completely degraded within a very short time.

Feeding trials in animal nutrition research have shown that amylomaize grain had no advantage as an animal feed compared to waxy or normal dent corn grain.[81] This may be attributable to amylose starch being less digestible than the amylopectin starch fraction found in normal dent and waxy corns.[82] Similar inferences could be made from data reported by Peters et al.[83] and Fergason et al.[84] when Angoumois grain moths, (*Sitotroga cerealella* (Oliv.)), were reared on high amylose corn grain. Larvae feeding on the high amylose kernels developed more slowly; subsequently, fewer moths reached the adult stage and weighed less than moths reared on normal dent corn. It was suggested that this insect could possibly be used to enhance selections for higher levels of amylose synthesis in a specially designed high amylose breeding project.[84] The data revealed nearly a 10% increase in amylose synthesis after seven cycles of recurrent selection when a heterogeneous composite sample of high amylose germplasm was exposed to the adult and larvae stages of the Angoumois grain moth.

New discoveries for the application of high amylose starch will continue to be found. Therefore, greater demand for high amylose grain will occur; however, the acreage devoted to high amylose grain production will probably remain relatively small compared with normal corn.

IV. WAXY CORN

A. Introduction

Variations in chemical and physical properties of corn endosperm are often identified by unique phenotypic expressions which are usually controlled by single recessive genetic factors. A corn selection found in China in the early 1900s was described as having an endosperm with a dull, waxy-like appearance.[85] This endosperm trait was subsequently found to be controlled by a single recessive gene on chromosome 9 and was designated as *waxy* (*wx*). It has also been referred to as *Chinese waxy* (*wx-c*) due to its origin of discovery. Coe et al.,[86] in a review of "The Genetics of Corn," emphasized the uniqueness of this phenotype and described the waxy trait as having a marble-like opacity with a hardness similar to normal corn. The distinctiveness of the waxy phenotype is so vivid that it is easily identified visually in most corn germplasm backgrounds; however, the moisture content of the kernel must be 16% or lower before the waxy trait can be recognized visually. Corn endosperms that are homozygous for the *wx* gene produce only the branched starch component (amylopectin), and are devoid of the linear amylose fraction. Another

unique characteristic of the waxy trait is its specific staining reaction when exposed to a dilute solution of iodine.[87] Amylopectin starch from homozygous waxy kernels forms an iodine-starch complex resulting in a reddish-brown stain. Alternatively, starch containing various levels of amylose or nonwaxy starch will stain blue to black when treated with potassium iodide. *Argentine waxy* (*wx-a*), an allele at the *wx* locus first reported by Andres and Basciollo,[88] is known to produce small amounts of amylose (<5%) and gives an intermediate staining reaction with iodine. Other mutant alleles at the *Wx* locus have been reported which possess similar starch properties to those observed with *wx*.[89,90]

References to the biosynthesis of amylose and amylopectin starch have been made earlier in this chapter and will not be covered again in this section. Since this section is devoted to waxy corn, no attempt will be made to elucidate on the various genetic interactions involving the *wx* gene and other mutants located at other loci in the corn genome. Research has shown that the *wx* gene is epistatic to all known endosperm mutants, thus accounting for the absence of amylose starch when the *wx* gene is present.[91,92]

B. Breeding Waxy Corn

From its introduction into the U.S. in 1909 and until the mid 1930s, the waxy gene was used primarily as a curiosity. Eventually, it came to be used universally as a marker in various genetic studies and experimental systems. By the early 1940s, work at the Iowa Agricultural Experiment Station revealed that the amylopectin starch from waxy corn had properties similar to tapioca starch obtained from roots of the cassava plant (*Manihot utilissima*).[93,94] Due to difficulties in importing tapioca from the Far East during World War II, the commercial production of waxy corn was begun. There was no apparent speculation at that time on the potential growth of this new specialty corn in either the U.S. or world markets. However, new discoveries for waxy starch applications over the last half-century has resulted in a substantial growth in acreage planted to waxy corn in the U.S., Canada, and Europe. This growth has been enhanced by the breeding program at the Iowa Agricultural Experimental Station. Most of the waxy corn improvements have been made by corn breeders within the private sector of the seed corn industry. Presently, there are probably fewer than six private seed companies devoting any significant breeding efforts to the development of waxy corn hybrids.

Unlike the complexities associated with hybrid improvements of high amylose corn, waxy corn breeding programs are generally more conventional and less laborious. This phenomenon is due to the unique expressivity of the waxy gene in corn germplasm and the ease with which it can be transferred between and within breeding populations. Emphasis is again placed on the unique staining characteristics of the waxy trait within the endosperm when phenotypic expression of the waxy gene is masked by other factors such as high moisture levels or aleurone color. The *wx* trait also may be easily classified in pollen grains by staining with a dilute solution of potassium iodide.[95] Pollen grains having only amylopectin starch stain reddish-brown while the non-waxy pollen grains containing amylose starch will stain dark blue to black. Heterozygous *Wxwx* plants should produce equal quantities of pollen grains of both genotypes. This pollen staining phenomenon makes it easier to select for the desired genotype in a breeding population prior to performing pollination procedures. This technique is especially helpful in a continuous backcrossing program designed to transfer the waxy gene in an inbred line-conversion program.

The breeding techniques or methodology a breeder might choose in any specific waxy development program is dependent upon many considerations including the breeding objectives, resources available, type of breeding populations or germplasm, level of expressivity of traits being selected, etc. The list of variables can be expanded considerably, but the breeder has to consider each program individually before choosing among the alternatives.

From the onset of many waxy inbred development programs, the backcross method has probably been the most popular. There is one compelling reason to choose this breeding method for waxy hybrid improvement. Competition among private seed companies to provide superior performing

waxy hybrids as fast as possible encouraged many breeders to rely upon the relatively simple backcross breeding method. Consequently, conversion of elite dent lines used in the best commercial dent hybrids would provide the fastest and probably the most positive results. There are limitations, however, imposed by the backcross breeding approach. New hybrid developments would theoretically be expected only to equal but not to exceed the performance of their normal dent counterparts and would probably be inferior to newer, more current dent hybrid developments. This breeding procedure necessitates that the waxy corn breeder choose alternative breeding methods designed to circumvent some of the apparent limitations imposed by the backcross conversion method. Initially, most private waxy breeding programs did not have adequate reserves of waxy converted germplasms to permit their utilization of more complex waxy breeding schemes. More flexibility within these programs has ensued as a few private waxy breeding programs have been expanded to increase their array of available waxy breeding populations. These programs should have advanced to the stage where breeding procedures could be pursued that are similar to those in some of the more competitive dent corn breeding programs within the seed corn industry.

It is not the intent of the author of this section to outline a breeding program for waxy hybrid improvements. However, the obligation exists to emphasize that breeding procedures applicable to waxy corn are, with minor exceptions, similar to those used in normal dent corn programs. The major requirement is the presence of the waxy gene within the source breeding population at a frequency that would provide a reasonable level of success in recovery, assuming proper selection techniques were used. For example, in a pedigree-selection scheme performed within a breeding population derived from crossing two elite inbred sources, it is necessary that only one of the two inbred lines contribute the waxy gene to the population. This is the only additional genetic trait, compared with a normal dent breeding program, that has to be considered in the selection process. Adequate sample size of the source breeding population is, however, of prime importance. Pollen staining of segregating or heterogeneous populations is a valuable technique in the selection phase of waxy inbred development programs. Hallauer et al.[96] implied that most applied corn breeders use similar procedures and perhaps similar germplasm sources in their dent inbred development programs. They also emphasized that minor variations between breeding programs exist including size of breeding populations, selection intensity, and subsequent testing procedures. Their discussions reveal the freedom that individual breeders have in choosing among many alternative procedures which they feel are most applicable to their own program, and those which may provide the greatest contribution to the success of their goals whether they be of short or long-term duration. The ultimate success of any well-designed program has been dependent upon the breeder's ability to select those progeny that will contribute in a positive direction toward the progress of the program.

Choice of germplasm must be the most critical for any corn breeding program. It is essential that selection of inbred lines to be crossed together for use in a pedigree selection scheme must complement each other for those attributes that contribute to desirable plant performance and grain yield. The choice of germplasm to be used in a breeding program is a decisive factor in determining the most logical breeding approach to be used. Normally, a combination of some scheme for population improvements in conjunction with inbred line development should be a part of most successful breeding programs.

Population improvement of germplasm breeding sources by some method of recurrent selection is just as applicable to a waxy breeding program as it is in normal dent programs. Most recurrent selection schemes, however, may be designed for a relative long-term approach and would require greater inputs of resources before any returns can be expected from a marketing aspect. Economics of a breeding program and the potential benefits from it have to be determined before making the final decisions on what approach to pursue. Waxy corn is considered a specialty type corn and does not have the market potential of normal dent; consequently, not all private seed companies involved in marketing waxy corn can justify an extensive breeding program including substantial expenditures into population improvement *per se*. Reconsiderations, however, are warranted if a private seed company has sufficient sales potential in both the dent and waxy corn markets.

The development of waxy inbred lines by self pollination within crosses among elite germplasm sources followed by selection and testing is a common procedure in most waxy corn breeding programs of the U.S. As indicated earlier in this section, the development of elite waxy inbred lines by the backcross method has been very successful. Regardless of the breeding methods used, waxy breeding programs can be enhanced by the employment of the pollen staining technique. This technique improves the efficiency of a program by eliminating pollination of plants which do not carry the *wx* gene. This technique is more applicable to consecutive backcrossing programs designed to obtain the maximum cycles per year and eliminates the necessity in a continuous backcrossing program for using test-crosses to detect plants having the waxy gene.

Progress in some waxy breeding programs has apparently been successful as revealed by the relatively competitive yield performance of waxy hybrids compared to normal dent hybrids. Yield comparisons among Custom Farm Seed waxy hybrids and their normal dent hybrid counterparts indicate yield differentials of 5% or less between the two types of hybrids (Table 3.2). Theoretically, there should be no expected yield differentials between a waxy converted hybrid and its normal dent counterparts. There is evidence available which indicates that waxy hybrids produce grain having a higher test weight than the corresponding dent counterparts (Table 3.3). Higher test weights should enhance the relative performance of waxy corn hybrids. There may be exceptions to comparative yields between waxy and dent hybrids if the breeder has not been effective in progeny selection while pursuing the backcross breeding program.

Most seed corn companies do not have a waxy corn breeding program, or if they do, it is an apparently small and insignificant part of their total breeding efforts. Nevertheless, there are some private corn breeders that devote a concentrated and substantial part of their breeding efforts to the continued improvement of waxy corn. These efforts, hopefully, will assure the commercial waxy grain producer that waxy hybrids should remain competitive in performance with the best dent hybrids.

TABLE 3.2
Relative Yield Performance of 5 Waxy Hybrids vs. Their 5 Dent Counterparts

		Dent version		Waxy version		
Hybrid	Number of comparisons	Moisture (%)	Yield (q ha^{-1})	Moisture (%)	Yield (q ha^{-1})	Difference (yield) (%)
A	20	22.2	100.3	23.3	94.8	–5.8
B	14	21.7	95.0	24.0	95.5	+0.5
C	18	22.3	103.3	23.4	96.2	–7.4
D	20	19.0	99.6	20.7	94.7	–4.9
E	12	20.7	85.0	22.0	85.3	0.3
	Avg.	21.2	96.6	22.7	93.3	–3.5

TABLE 3.3
Comparative Test Weights of 14 Waxy Hybrids and Their Normal 14 Dent Counterparts from 2 Locations in 1982

Grain type	Number of comparisons	Test weight[a] (kg/M)
Waxy	28	751
Normal dent	28	736

[a] Measurements on F2 grain samples stored for 1 year under comparable conditions.

C. Production and Management of Waxy Corn

Waxy corn production, like high amylose, is controlled by a single recessive gene; therefore, seed production and quality assurance procedures for maintaining genetic purity should be very strict. It is essential in foundation seed, commercial seed, and commercial grain production fields that adequate isolations are maintained to avoid cross contamination arising from foreign or nonwaxy pollen. Most seed corn companies that produce waxy hybrid seed have isolation requirements sufficient to prevent undesirable outcrossing to nonwaxy sources. They should also have specified tolerance limitations for seed purity standards. For example, the quality assurance program of Custom Farm Seed specifies that foundation waxy seed used for commercial seed production must be at least 99.5% pure waxy seed. That factor in conjunction with strict isolation permits Custom Farm Seed to label all commercially sold waxy hybrid seed as being at least 97% pure waxy. All seed fields are thoroughly and systematically sampled prior to harvest to assure the company of maintaining these standards. Any portion of a seed field that does not meet these rigid standards is not harvested for seed processing.

A majority of the commercially produced waxy grain is produced under contract to wet milling companies. The contact agreements, in addition to specifying certain grain quality standards, also have waxy purity requirements of the waxy grain. It is obvious why these strict standards must be adhered to in the corn wet milling industry. Variations in the amylopectin components could result in failures by the wet milling processor to meet specific standards of the processed starch. Premiums are paid to the waxy grain producer by the wet miller or the waxy grain exporter as compensation for the extra quality control procedures that must be followed. Other than the prior-mentioned quality assurance requirements for waxy grain production, similar cultural practices followed in normal dent grain production are adequate for managing waxy grain production. Responses to fertility applications, diseases, insects, environmental stresses, etc. theoretically should not differ between waxy hybrids and their normal dent counterparts because no physical or chemical differences should exist between these putative isogenic counterparts except in the kernel. Sucrose is the sugar of transport, but it is not normally converted into starch until it is translocated to the corn endosperm.

Only a relative few seed corn companies produce or merchandise waxy hybrids. Acreage devoted to waxy corn production probably makes up less than 1% of the total corn acreage in the U.S. Only those companies having a large proportion of this market can economically justify being in the waxy seed business. Information available from advertisements and product literature from various seed corn companies reveals that at least a dozen of them offer waxy hybrids for retail sales. Most of these companies offer fewer than five waxy hybrids covering a very narrow range in relative maturity. An exception is Custom Farm Seed of Momence, IL, which is recognized in the seed corn industry as a company very extensively involved in specialty corn research and development. At the time of the writing of this chapter they offered 20 waxy hybrids ranging in relative maturity from 83 to 122 days.

Most of the waxy grain produced in the U.S. for industrial utilization is under contract to three wet millers: National Starch & Chemical Company, A. E. Staley Company, and American Maize Products. These companies normally contract directly with waxy grain producers to fulfill their requirements for specialty hybrid grain. The National Starch and Chemical Company, which is also a large consumer of high amylose grain, would be considered as the largest wet milling processor of specialty grain. Their affiliation with Custom Farm Seed has resulted in the only fully integrated combination of seed producer and consumer within the U.S.

In addition to waxy grain being produced in the U.S. for the wet milling industry, there is additional acreage of waxy grain grown for export and/or for use as feed in the livestock, dairy, and poultry industries.

D. Utilization of Waxy Corn

Wet milling of corn grain is a process used to separate starch from other constituents, such as fiber, germ, protein, and other minor extraneous materials.[1] The separation is enhanced by steeping or soaking grain in warm water containing sulfur dioxide. After the starch is separated from the other components, additional steps in the extraction of pure starch are required, including screening, washing, and eventually drying.[97]

The recovery of waxy starch is only the first step in a multitude of possible treatments or modifications that starch can be subjected to prior to its utilization in food or industrial applications. The unique properties of waxy cornstarch compared to normal dent corn is partially attributable to the absence of the amylose fraction present in dent corn. Additional properties such as degree of branching and length of side branching of the starch polymer also have an impact on determining uniqueness within waxy starches. The large size of the amylopectin molecule and its branched nature reduced the tendency for amylopectin polymers to form hydrogen bonding and subsequent retrogradation.

All plant starches are unique due to their specific chemical and physical properties. Most starches in their native or unmodified form have limited use in various industries. Therefore, most starches including waxy cornstarch are modified either to improve or repress their inherent properties as may be required for special use applications. For example, the general procedure of cross-linking waxy starches through the use of various chemical reagents improves the integrity of starch granules by reinforcing the hydrogen bonds in the granule, thus inhibiting swelling and eventual rupturing of the granule *per se*. Many types of modified waxy starches have a multitude of applications in the paper, textile, corrugating, and adhesive industries in addition to an enormous array of applications in the food industry.[1,69,70,72–77,98]

Modified waxy cornstarches serve essential functions in foods, including the improvement of uniformity, stability, and texture in various food products. The apparent benefits from these functions include the improvement of smoothness and creaminess in canned foods and dairy products, better freeze-thaw stability in frozen foods, and more desirable texture and appearance in dry foods and mixes.

Major advancements in papermaking technology are in part related to the availability of new types of modified waxy starches. These starches essentially provide binding or bonding qualities to the papermaking process, as well as adding other essential features, including improved sizing for greater paper strength and printing properties. Papermaking is a very complex and highly technical science which cannot be adequately covered in the space allocated to this chapter. Progress in papermaking technology is occurring rapidly and is therefore adding substantially to the wealth of information previously available.

Waxy cornstarches also are used in various textile finishing processes because of their clear film forming properties.[74] The unique properties of waxy cornstarch provide for additional application in the textile, corrugating, and adhesive industries.[69,72,77] Waxy cornstarch is a major starch component in adhesives used in making bottle labels. This waxy starch based adhesive imparts resolubilizing resistance to the labels which prevents their soaking off the bottle if immersed in water or being subjected to very high humidity conditions. In addition, waxy cornstarches are commonly used in the manufacture of gummed tapes and envelope adhesives.

An alternative to the use of waxy starch in various industrial and food applications is the use of waxy corn grain as livestock feed, which began to emerge in the 1940s. The use of corn in the feed industry has always held a lofty position because of its value in producing meat, milk, and eggs. Beginning with a research report in 1944, waxy corn seemed to have the potential to increase feed conversion efficiencies.[99] Additional feeding trials involving swine, beef and dairy cattle, lambs, and poultry were designed to compare the feeding value of waxy to normal dent grain.[81,100–102] Generally, the trials indicated an advantage for feeding waxy grain. Seldom have the investigations shown any negative or adverse effects from feeding waxy grain. Testimonials that indicate increases

of both milk production and butterfat content are not uncommon when waxy corn is fed to lactating dairy cattle. Increases of more than 20% in average daily weight gains in fattening lambs were observed when waxy grain was compared with normal dent.[102] In addition, a 14.3% increase in feed efficiency was noted in favor of waxy grain. Likewise, an increase in feed efficiency approaching 10% was obtained in trials where waxy grain was compared with the dent counterparts when fed to finishing beef cattle.[102] Lower back fat and higher carcass grades have been reported in swine-feeding experiments (unpublished testimonials). As a result of the generally favorable advantages associated with feeding waxy grain, many livestock producers and dairymen use waxy corn as a replacement for normal dent in animal diets. Increased digestibility of the waxy starch may explain these advantages attributable to the use of waxy corn as a feed. Conversely, amylose starch present in normal dent corn has been suggested as being less digestible in studies performed on monogastric animals.[82] Since the amylose starch component is absent in waxy corn, this may add credence to evidence that waxy corn has been shown to be superior in animal diets.

Though the history pertaining to waxy corn and to its utilization is relatively brief, the cornstarch industry can be assured that the demands for this unique polymer will continue to expand, but at an unknown rate. World population growth, changes in modern living styles, the desire for more human conveniences, and progress in various industrial and food technologies will surely lead to greater demands on specialty polymers including waxy and amylose types.

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4 High Quality Protein Corn

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I. INTRODUCTION

The need to improve the nutritional value of corn (*Zea mays* L.), or maize, as it is often called in different parts of the world, has been recognized for a long time. In the mature corn kernel, the two principal components, namely, the endosperm and the germ, contain most of the kernel protein. The germ protein is superior in both quality and quantity. In contrast, the endosperm protein is not only low in quantity but also has poor quality. The relative amounts of protein contributed by the endosperm and germ vary and are dependent on the type of corn, genotype, texture, and size. In most field corns, the endosperm accounts for 80 to 85%, and the embryo accounts for about 8 to 10% of the total kernel dry weight.[1] Although there is variation among different classes of corn, genotypes, and environments, it is estimated that endosperm may contribute as much as 80% of the total kernel protein.

Concerns regarding poor nutritional value of corn have been expressed in various national and international organizations. The nutritional problems, however, are complex and lack consensus. Breeding goals are more difficult to achieve, requiring long-term investments, sustained research efforts, patience, and containing administrative, financial, and scientific support. Genetic manipulation of nutritional goal(s) requires no yield penalty, which is often difficult because of the negative correlation between yield and such traits. The only exception(s) occur when there is a commodity-added value for such a product both at industrial and consumer levels.

Attempts to improve protein in corn began in the last part of the 19th century. The quality, however, became more of a concern at a much later date. A continuous search has been made for mutant alleles that would possess a better protein quality in terms of lysine and tryptophan, the two limiting essential amino acids in the corn endosperm protein. The history stands testimony that maize breeders are always screening for such spontaneous discoveries that will facilitate their task to improve needed attributes, It should, however, be cautioned that such genes and gene combinations are, more often than not, difficult to exploit in a satisfactory manner because of negative associations. The end result is generally frustrating, thus culminating efforts partially or sometimes completely to continue further research. Also, as problems become more apparent and the objectives seem more difficult to achieve, the financial support starts dwindling, thus affecting research efforts adversely and further slowing down the progress.

The historical aspects of high quality protein corn (HQPC) dates back no more than three decades. The enthusiasm and great optimism to improve the quality of protein in corn had its beginning with the discovery of high lysine gene opaque-2 (o_2)[2] and floury-2 (fl_2)[3]. These mutant alleles were not new variants because they were known much earlier and were used as genetic markers. The opaque-2 gene was first discovered by Singleton and Jones and reported by Emerson et al.[4] The floury-2 gene was first discovered by Mumm as reported by Emerson. Since then the search for new variants has continued, and we have additional mutants that will be discussed later. It is of course important to mention that of all the known mutants, the two that have been tried and used extensively are opaque-2 and floury-2 in breeding programs around the world. Though floury-2 was used initially, its use has been practically abandoned in the recent years. The investigations and research conducted do not offer any better alternative to the opaque-2 gene.

II. RELATIVE ECONOMIC VALUE OF CROP

The area planted to opaque-2, modified opaque-2 (Quality Protein Maize), and other HQPC and the resulting production has varied from year to year since the discovery of the high lysine genes. No definite statistics were ever available except in some countries. Also, in the past three decades, there have been shifts in emphasis and production in different countries. Some of the countries that had embarked very aggressively on the opaque-2 corn have practically scaled down the production of this type of corn. At one time a sizable volume of opaque-2 corn was being produced in the U.S., Brazil, Columbia, and perhaps in some east European countries, but it is not true any more except in Brazil. Many countries in the world still manifest continuing interest in this type of corn but unfortunately lack the financial resources to conduct breeding programs with moderate- to well-established backup laboratory analysis facilities. Given adequate research funding, many countries can benefit and directly exploit germplasm that has been developed at Centrto Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) over the past two decades. To date, no reliable statistics are available on the acreage and production but at least some countries, Brazil, China, U.S., Guatemala, Ghana, and South Africa, among others, have sufficient HQPC. It is estimated that farmers in the U.S. midwest produce one million tons of Crow's Hi-Lysine corn annually all of which stays on the farm to be fed to the livestock, mainly growing and finishing pigs. Areas devoted to this type of corn also include Peru, Bolivia, Ecuador, Vietnam, and Senegal. Global 2000 is placing a major thrust in promoting this corn in Ghana and other African countries, and is in the process of rapidly multiplying seed to cover several thousands of hectares. Considering total corn production in the developed and the developing world, this corn is still relatively insignificant compared to the normal endosperm corn cultivars.

III. BIOCHEMICAL CHARACTERIZATION AND GENETIC BASIS

The maize genome possessing only ten pairs of homologous chromosomes is richly endowed with a whole array of endospermic mutants that can modify protein, starch, and oil characteristics of the mature corn kernel, particularly the endosperm. The variants already known that affect endosperm characteristics are numerous. Of particular interest in this chapter are mutants that affect protein quality. In regards to protein content, no specific mutants have so far been detected or reported in literature. Interesting work, however, has been conducted in several institutions, especially at the University of Illinois. It is not intended in this chapter to have detailed discussion on this topic. Only quality aspects in relation to protein quantity will be discussed in appropriate sections. The readers interested in breeding for protein content should consult excellent reviews available on this subject.[5–7]

A. High Quality Protein Mutants

Several mutants have been detected over the past 30 years than can favorably modify characteristics of the corn endosperm protein by elevating levels of two deficient amino acids, namely lysine and tryptophan. The value, use, and inheritance characteristics of such genes, however, vary tremendously. The first discovered high lysine mutant was opaque-2 (o_2).[2] Shortly thereafter the biochemical effects of floury-2 (fl_2) mutant were discovered.[3] Search for new and better genes has continued, and to date, additional mutants are known that can improve protein quality of corn endosperm protein. Some such mutants worth mentioning are opaque-7 (o_7),[8,9] opaque-6 (o_6),[10] floury-3 (fl_3),[10] mucronate (Mc),[11] and defective endosperm (De-B30).[12] Attempts also have been made to search high lysine gene(s) but with still a high level of zein fraction. Two such mutants, opaque 7749 and opaque 7455 (o_{11}), were identified by Nelson.[13] The opaque 7749 is particularly interesting since it is markedly higher in lysine than the normal counterpart, although not as high as opaque-2, and the prolamin fraction is quite high. The mutant 7455 was later referred to as opaque-11.

B. Changes in Amino Acid Profile

The effects of HQPC mutants have been studied by several research workers worldwide. Of course more studies have been conducted with o_2 and fl_2 mutants and thus much of the available information in literature is available on these two mutants. Both o_2 and fl_2 mutants change the amino acid profile, thus resulting in an increase of lysine and tryptophan. The lysine in corn is the first and tryptophan the second limiting amino acid. In addition, other amino acids such as histidine, argenine, aspartic acid, and glycine have shown an increase. Some other amino acids like glutamic acid, alanine, and leucine are decreased compared to normal corn. A most notable decrease occurs in leucine. This is desirable because it makes the leucine-isoleucine ratio more favorable, which in turn helps to liberate more tryptophan for niacin biosynthesis. HQPC has notable benefit in combatting pellagra, even though it has no more niacin than normal corn. Methionine, a sulfur containing amino acid, registers an increase in fl_2, o_7, and o_6. Other mutants, however, show no change in the content of methionine.

C. Alterations in Protein Fractions

Corn endosperm protein is comprised of different fractions. Based on their stability, these can be classified into albumins (water soluble), globulins (soluble in saline solution), zein or prolamine (soluble in relatively strong alcohol), and glutelins (alkali soluble).[14] In normal endosperm, the proportion of various fractions on the average are albumins 3%, globulins 3%, zein 60%, and glutelin 34%. On the other hand, the embryo protein is predominantly in the form of albumins (+60% of total embryo protein). The alcohol-soluble fraction accounts for only a small percentage (5 to 10%).[15–17]

The prolamine fraction (zein in corn) is low in lysine content, containing only 0.1 g/100 g of protein. This fraction itself cannot support the growth of rats.[18,19] The addition of tryptophan and lysine (0.5%) resulted in nearly normal growth of rats. The other amino acid tryptophan is also low in zein. The glutelin fraction in corn is considerably higher in lysine, exhibiting levels of 3 to 2 g/100 g protein or even higher.[20,21]

The desirability of reducing zein content as a means to improve the protein quality of corn endosperm has long been recognized.[22,23] It was felt that if zein fraction could be reduced, or stopped completely, the other fractions will be formed in much larger proportions. Since non-zein fractions are higher in lysine and tryptophan, their increase would result in enhancing lysine content in the mutant kernels. It had also been hypothesized at one time that mutations giving the opaque phenotype were most likely to be deficient in zein. This presumption, however, was not true because there are several mutants with an opaque phenotype which do not necessarily have reduced zein or high lysine and tryptophan such as opaque-1 (o_1) and floury-1 (fl_1).

The introduction of high quality protein corn mutants alters the relative amounts of four major protein fractions present in corn protein. The zein fraction is markedly reduced by roughly 50% with a concomitant increase in the relative amounts of albumins, globulins, and glutelins. The protein fractions of normal and opaque-2 corn analyzed by Landry and Moureaux procedure[24] indicates that fraction II (true zein) is reduced whereas fraction I and V are increased. From the foregoing it is apparent that HQPC mutants increase the levels of lysine and tryptophan by suppressing synthesis of lysine-deficient zein fraction. Since fractions other than zein are higher in lysine and tryptophan, zein reduction causes proportional elevation of other fractions high in lysine. The result is that the levels of lysine and tryptophan become elevated in protein, but not on absolute basis of per unit endosperm.

D. Inheritance

The specific chromosome and position on the chromosome is known for some of the mutants. The o_2 mutant is located on chromosome 7 (position 16), fl_2 on chromosome 4 (position 63), opaque-7

on chromosome 10 (position 87), floury-3 on chromosome 8 (long arm), and De-B30 on chromosome 7 (short arm). The mutants o_2, o_6, o_7, and o_{11} are completely recessive and manifest their biochemical effects on zein synthesis only when present in homozygous recessive condition. The two floury mutants, fl_2 and fl_3, are semi-dominant and exhibit variable expression for kernel opacity and protein quality depending on the presence of one or more recessives in the triploid endosperm. The mutant De-B30 behaves like a dominant in regards to showing dosage effects on kernel opacity and zein content.[25,26] The Mc allele is dominant in reducing zein content and is aspecific to zein subunits.[11] Except opaque-6, considered as a structural gene, the others are regulatory genes. All these mutants have several common features, including low prolamine protein fraction, soft chalky endosperm, and a deficiency in the amount of dry matter produced.

E. Pleiotropic and Other Secondary Effects

It is well known that new genes and gene combinations that bring about drastic alterations in either plant or kernel characteristics also produce several secondary or undesirable effects. The low prolamine or high lysine mutants are no exceptions. These mutants, besides reduced zein synthesis and altered concentration of lysine and tryptophan, also affect several other biochemical changes which are presented in review articles by several authors.[27–32] The information presented therein indicates that in general, o_2 has higher ribonuclease activity compared to normal, reduced glutamate dehydrogenase, alterations in trypsin inhibitor, and changes in several soluble proteins.

In addition to biochemical traits, the high lysine mutants adversely affect a whole array of several agronomically important traits including kernel characteristics. The poor agronomic performance of HQPC has been reported by several research workers.[31–44] The o_2 and other mutants affect dry matter accumulation adversely, thus resulting in lower yields due to increased endosperm size. The kernel phenotype is also changed to a soft chalky dull appearance not liked by many growers in the developing countries. The kernels dry slowly following physiological maturity of the grain and have a higher incidence of ear rots. Potassium, zinc, and oil content may show an increase in opaques over the normals. Other changes include thicker pericarp, larger germ size, reduced cob weight, reduced color intensity in yellow corns, and reduced kernel weight and density. Such effects may, however, differ in different genetic backgrounds and sometimes within the same background, especially if the material is structured on a family basis. Genetic background effects could play a very important role in selecting for desirable genotypes. A more detailed discussion of pleiotropic effects and manipulating them to a breeder's advantage is presented in later sections.

F. Modifying Genes

Modifying genes or genetic modifiers are a series of genes which apparently do not have any effect of their own but they do interact and modify the expression of quality protein corn mutants. The effect could be on any trait, but more pronounced changes have been observed in regards to kernel phenotype. Though any quality protein mutant could be involved in an interaction with a modifying gene complex, the greatest effort has been spent on the opaque-2 gene. The reason is obvious, as no other mutant offers any additional advantage over the opaque-2 gene system. Its inheritance is simple and not complicated by any dosage effect for kernel opacity or protein quality.

The role of genetic modifiers in altering kernel phenotype has been studied much more extensively than any other trait. The modified opaque-2 kernels have been observed and studied by several breeders working on quality protein breeding programs.[45–47] The pattern of kernel modification can be either regular or irregular. In regular patterns, the modified fraction increases progressively from the crown towards the base of the kernel. In irregular patterns, the translucent fraction may be present as a band, scattered, resembling a bridge, and translucent base. From a practical standpoint, only regular patterns appear more important and have been emphasized by most corn breeders. Various aspects of genetic modifiers have been discussed in earlier publications[40–42,44,57] and only salient features will be discussed. Quality consideration of genetically

modified opaque-2 kernels is extremely important. Several reports indicate that soft opaques and modified opaques do not differ in protein quality.[45,51,54,55] However, experience of CIMMYT scientists and that of other research workers is quite contrary to the above findings. Protein quality and kernel modification or vitreousness generally are negatively correlated. Exceptions, however, occur when protein quality of the samples is monitored. The results may be frustrating initially, but as the accumulation of favorable modifiers continues, fewer samples in each generation would need to be discarded. It has been amply demonstrated that good kernel modification and acceptable protein quality can be combined. Perhaps in some materials one may have to sacrifice a slight decrease. Protein content of modified opaques registers a slight increase.[40–42] Even in individual modified kernels the vitreous fraction is generally higher in protein content compared to the soft fraction. Alterations in kernel modification also reveal interesting changes in the protein fractions. Though different fractions may be altered differently, there is a general tendency for the zein or prolamine fraction to increase, thereby slightly lowering the protein quality. The other fractions which are good in protein quality may register slight or no change. Variable trends have been observed in different hard endosperm opaque-2 materials.

Accumulation of genetic modifiers changes physical characteristics of opaque-2 kernels with respect to vitreousness. This may or may not always be reflected in increased kernel density and weight. Variation and opportunities for selection do exist in materials undergoing such selection process resulting in better performing materials approaching standard genotypes.[40,58] The expression of genetic modifiers may be affected by maternal influence. Since endosperm is a triploid tissue, one may expect maternal influence since two doses of modifying alleles are contributed by the maternal parent and only one by the paternal parent. Reciprocal differences in crosses between soft opaques and modified opaques have been reported by some workers. Other factors such as genetic background and kernel texture could also alter phenotypic manifestation of modifying genes. Flint genetic backgrounds generally exhibit a higher frequency of modified kernels.

The information on the inheritance of genetic modifiers is limited. Several different types of genetic modifiers are likely to exist and perhaps do exist in different genotypes. The rich heterozygosity and heterogeneity endowed in the corn genome has helped to create an enormous genetic variability and diversity over thousands of years which is well represented in about 250 races of corn recognized to date.[59,60] The evolutionary process has continued and the genotypes in different ecological niches around the world have accumulated arrays of different modifiers favoring the dominant allele of the opaque-2 locus. To reverse the evolutionary process would require a great deal of slow, painstaking, and evolutionary work.[33] One would therefore expect that the genetic system controlling the modifiers be simple, moderately complex involving a few genes, and perhaps highly complex. A dominant suppressor gene has been reported by Pollacsec.[61] The homozygous opaque-2 dominant kernels of this suppressor gene were in most instances practically normal in texture. The heterozygous kernels for this suppressor gene exhibited floury mottling. Other researchers have indicated involvement of only a few genes in kernel modification.[62–66] The work at CIMMYT and at other institutes has indicated quantitative nature of modifying gene system.[39–42,55,67–69] The additive genetic variation seems to be more important in controlling kernel vitreosity in opaque-2 corn. Glover,[32] Glover and Mertz,[31] and Bjarnason and Vasal[44] have reviewed several studies which show that the modified texture of modified kernels is governed by the quantitative genetic system with additive gene effects playing an important role in controlling this trait; however, in other cases either a single gene or perhaps a few genes may be involved in kernel modification. Single gene modifiers will have a definite role in conversion programs provided protein quality is maintained and that they can facilitate in overcoming problems associated with the opaque-2 system. CIMMYT experience in this regard is quite extensive and is based on working on a wide range of materials extending over a period of over 20 years. One cannot discredit the existence of simply inherited modifiers or modifiers with few genes, but complex problems encountered in opaques warrant their limited practical use, if at all, if such problems are to be resolved satisfactorily.

G. Biosynthesis and Genetic Regulation of Storage Proteins in Maize

The increase of two amino acids, lysine and tryptophan, in the opaque-2 corn is brought about by an alteration of the relative amounts of different fractions constituting the corn endosperm protein. The zein or prolamine fraction is reduced substantially, thus increasing the relative amounts of other fractions in relation to this fraction. Since zein is practically devoid of lysine, while other fractions are rich in lysine, the overall amino acid profile shows an increase in lysine. It may, however, be remembered that no new proteins are formed and that the composition of various protein fractions remains unaffected. An increase in protein quality requires reducing the zein fraction. In addition to changes in the distribution of protein fractions, other effects have been observed. The ribonuclease activity is several times higher in opaque-2 compared with normal,[70,71] whereas floury-2 fails to induce any change in this respect. This activity cannot be regarded as the principal factor for reducing zein accumulation in the endosperm but instead a secondary effect associated with opaque-2 mutation. Also, the mutants affect differently the various groups of polypeptide or components or subunits making up the zein fraction. The subunits of zein fraction are altered differently by different genes. The SDS-polyacrylamide gel electrophoresis reveals that zein of normal corn contains six separable components, such as Z_1, Z_2, Z_3, Z_4, Z_5, and Z_6. The Z_1 and Z_2 are the two predominant types with molecular weights of 21,800 and 19,000 daltons and are rich in glutamic acid, leucine, and proline, but low in lysine. Of the four minor bands, Z_3, Z_4, Z_5, and Z_6, the latter two exist in only trace amounts. The non-allelic high quality mutants suppress one or more of these polypeptide bands. The opaque-2 particularly suppresses the synthesis of Z_1 whereas the floury-2 affects suppression of both major polypeptide components Z_1 and Z_2. The opaque-7 strongly suppresses the synthesis of Z_3 and Z_4 while strongly reducing Z_2. The defective endosperm De-B30 affects only Z_1 subunits.[72] The mutant o_6 is unique in suppressing synthesis of all subunits. In two mutant combinations, o_2 is epistatic to fl_2[73,74] while o_7 is fully epistatic to fl_2.[75] The mutants o_2 and o_7 are synergistic with Mc in controlling zein deposition. An additive effect is noted with o_2 and o_7 in suppressing zein synthesis.[74,76]

There are differences in developmental rates and levels. Both o_2 and o_7 have the lowest rate of zein accumulation during development, whereas fl_2 shows intermediate levels between opaque mutants and normal. The opaque-2 endosperms may have higher, lower, or unaffected levels of several enzymes involved in N[77] or carbohydrate metabolism.[78,79] Alterations in the levels have been observed for several enzymes including trypsin inhibitor.[80–82] glutamate dehydrogenase,[83] and RNase polymerase II.[84] The alteration of RNase polymerase could satisfactorily account for the variation in protein synthesis. This observation suggests that the effect of opaque-2 mutation may be at the level of transcription.

IV. GERMPLASM AND SOURCE MATERIALS

The discovery of the biochemical effects of the HQPC mutants spurred enthusiasm and led to a worldwide effort of converting elite inbreds and open-pollinated cultivars to opaque-2 and floury-2. Efforts were initially concentrated at Purdue University, but as donor stocks carrying these genes were made available, concerted conversion programs were undertaken by most corn breeders in practically every country. Mutant genes were introduced into a wide array of genetic backgrounds. Within a span of only a few years, a vast volume of HQPC germplasm was developed. The HQPC varieties and hybrids went into commercial production in some countries but were withdrawn because of lack in yield competitiveness and other problems in agronomic performance. Interest started to decline and financial support became more limited with the result of most programs starting to reduce or to abandon this activity. Over the years, therefore, much of the germplasm that was developed was lost. Countries, institutions, and private companies having good cold storage facilities may still be storing this germplasm. Fortunately, some institutions have maintained sustained efforts in the breeding of HQPC. A wide variety of germplasm is available including soft

opaques, modified opaques (QPM), QPM combining high oil characteristics, sugary-2-opaque-2 double mutant combinations, and fl_1o_2 combinations.

The soft opaque-2 germplasm is available in the form of broadbased populations, synthetics, o_2 converted lines and hybrids. CIMMYT converted several genotypes to soft opaque-2 types, but now has only a few soft o_2 populations because of continuing emphasis on developing hard endosperm opaque-2 genotypes. Some of the populations of interest will include Tuxpeño opaque-2 (Population 37), CIMMYT opaque-2 composite, Composite 1 (highland adaptation), Composite K (tropical adaptation), and Puebla opaque-2 composite. The University of Illinois, Urbana, has developed some synthetic populations which they have used for recurrent selection studies,[62] including SSSS-o_2, disease oil synthetic (DO-o_2), Syn. A-o_2, and Syn. B-o_2. Purdue University developed Temp. HA-o_2, Temp. HB-o_2, and colus synthetic. Another breeding population BSAA-o_2 was developed in Iowa by Loesch.[85] BSAA-o_2 is an improved opaque-2 version of broadly based Iowa synthetic AA (BSAA). Superior 10% of S_1's for yield, percentage protein, and percentage of lysine were recombined to form BSAAo_2-C1. The All India Coordinated corn breeding has three breeding populations (Shakti, Rattan, and Proteina) that were released in 1971. The national corn and sorghum program of Thailand has opaque-2 versions of Thai opaque-2 composite and Thai opaque-2 composite #3. In addition to soft opaque-2 breeding populations, opaque-2 versions of elite normal lines are available at Purdue (Oh.7, B14, B37, Oh.73, HS5, H60, W64A, C103, C123, R177, R181, A545, A619, and A632), Nebraska (N6, N6G, N13, N15, N31), Italy (Lo876 o_2, Lo888 o_2, Lo894 o_2, Lo908 o2, Lo968 o_2 and Lo970 o_2), Republic of South Africa [(B) 28W, BO 42W, BO 46W, BO 59W, BO 155W, BO 163W, BO 165W, DO874, white opaque-2 lines and B312Y, B451Y, BO385Y, BO394Y, BO395Y, BO404Y, BO412Y, BO461Y, DO940Y-1, DO940Y-3, HO4664, and HO467Y as yellow opaque-2 lines]. In addition, 22 released lines in Rumania and 8 in Yugoslavia are available. China has several soft opaque-2 lines which are being used in the production of soft o_2 hybrids. Zhong Tan #206 and #209 are now planted in 70,000 ha. Perhaps Colombia and Brazil also have some o_2 lines which they used at one time in hybrids H208, H255, and Ag502, and Ag504. There are reports of opaque-2 lines and hybrids developed in eastern Europe and Russia.

As regards modified opaque-2 types, CIMMYT has perhaps the largest collection. Most of this germplasm was developed and improved over a span of almost 20 years. The materials developed at CIMMYT include several QPM populations and pools which are listed in Table 4.1 and Table 4.2. These breeding populations possess different ecological adaptation, maturity, grain color, and texture.

Crow's Hybrid Seed Company in the U.S. has perhaps the more extensive program to develop QPM germplasm. They have used CIMMYT QPM populations and a Russian line in developing

TABLE 4.1
QPM Populations and their Characteristics

Population Number	Name	Adaptation	Maturity	Seed color	Seed texture
61	Early Yellow Flint QPM	Tropical	Early	Yellow	Flint
62	White Flint QPM	Tropical	Late	White	Flint-Semiflint
63	Blanco Dentado-1 QPM	Tropical	Late	White	Dent
64	Blanco Dentado-2 QPM	Tropical	Late	White	Dent
65	Yellow Flint QPM	Tropical	Late	Yellow	Flint
66	Yellow Dent QPM	Tropical	Late	Yellow	Dent
67	Templado Blanco Cristalino QPM	Subtrop.	Interm.	White	Flint
68	Templado Blanco Dentado QPM	Subtrop.	Interm.	White	Dent
69	Templado Amarillo QPM	Subtrop.	Interm.	Yellow	Flint
70	Templado Amarillo Dent QPM	Subtrop.	Interm.	Yellow	Dent

TABLE 4.2
QPM Gene Pools and Their Characteristics

QPM pool Number	Adaptation	Maturity	Seed color	Seed texture
Pool 15 QPM	Tropical	Early	White	Flint-Dent
Pool 17 QPM	Tropical	Early	Yellow	Flint
Pool 18 QPM	Tropical	Early	Yellow	Dent
Pool 23 QPM	Tropical	Late	White	Flint
Pool 24 QPM	Tropical	Late	White	Dent
Pool 25 QPM	Tropical	Late	Yellow	Flint
Pool 26 QPM	Tropical	Late	Yellow	Dent
Pool 27 QPM	Subtropical	Early	White	Flint-Dent
Pool 29 QPM	Subtropical	Early	Yellow	Flint-Dent
Pool 31 QPM	Subtropical	Intermediate	White	Flint
Pool 32 QPM	Subtropical	Intermediate	White	Dent
Pool 33 QPM	Subtropical	Intermediate	Yellow	Flint
Pool 34 QPM	Subtropical	Intermediate	Yellow	Dent

this type of germplasm. Purdue University, Texas A&M, and University of Illinois have also used CIMMYT QPM germplasm in developing source breeding populations and lines adapted for U.S. conditions. Good modified opaque-2 germplasm has also been developed in South Africa, in addition to two good source populations POWS1 (Modified White opaque) and DOYS (Yellow modified opaque) developed for the purpose of producing modified opaque-2 hybrids. Both these populations have good combining ability and good disease tolerance. Chinese scientists are also interested in developing hard endosperm o_2 germplasm. The Chinese are using germplasm that was developed at CIMMYT and are extracting lines from CIMMYT populations 70, 41, pool 33 QPM, and Tuxpeño QPM. Recently Brazil has also become deeply involved in breeding QPM genotypes. They are, however, using germplasm developed at CIMMYT. Ghana-Sasakawa Global 2000 has recently become involved in QPM. They are improving QPM populations, developing lines, and producing experimental hybrid combinations using germplasm developed at CIMMYT. The Italian group[68] has developed a modified opaque-2 breeding population which is called MOD2-C5. This was developed from crosses among highly modified o_2 inbreds. Selection has continued for 5 cycles for improving the degree of modification of the opaque-2 hybrids.

Efforts also have been directed to develop sugary 2-opaque-2 germplasm. Much of this germplasm has been developed and studied intensively at Purdue University. The double-mutant combinations are available in some inbreds. CIMMYT's breeding effort in developing this combination has been quite modest. Several tropical and subtropical QPM populations were converted to su_2o_2. Selected families with good kernel modification, kernel size, and color were used in synthesizing su_2o_2 composite. It is a broadbased breeding population possessing modified endosperm with deep yellow kernels, more number of rows and kernels per ear.

Germplasm with o_2 gene in floury-1 background has been developed in Bolivia. Several breeding populations carrying the o_2 gene in floury-1 backgrounds have been developed. One variety, Aycha Sara 5, is commercially planted in highlands. CIMMYT has devoted some effort to develop such a combination. The o_2fl_1 conversions of two highland pools 3 and 8 were developed and improved for some time. Later, two fl_1o_2 composites were developed and designated early floury opaque-2 and later floury opaque-2 composites.

Sources germplasm carrying floury-2 gene is limited because most breeders abandoned its use since 1975. Two breeding populations carrying this gene have been developed at the University of Illinois; these populations are SSS-fl_2 and DO-fl_2.

In addition to source endosperm, CIMMYT has recently developed several modified phenotype o_2 lines. These lines carry CML numbers from 140 to 194 (Table 4.3).

TABLE 4.3
Key Characteristics of QPM Tropical Inbred Lines Available from the CIMMYT Corn Program Mexico, 1992

Line number	Pedigree	Maturity	Color	Texture	Protein in grain (%)	Tryptophane in protein (%)	% of mean yielda 1990 trialb	
							Tester 1	Tester 2
CML140	P62c3HC87-2-1-#-#-1-B-#	Late	White	Flint	10.8	1.06	95	103
CML141	P62c5HC24-5-2-3-1-B-B-2-B-B-#	Late	White	Flint	8.6	1.05	–	–
CML142	P62c5HC93-5-6-1-3-B-B-B-7-B-B-#	Late	White	Flint	10.2	1.03	82	99
CML143	P62c6HC88-1-1-B-B-B-10-B-B-#	Late	White	Flint	10.7	0.89	103	101
CML144	P62c5HC182-2-1-2-B-B-3-1-#-#	Late	White	Flint	10.5	1.02	–	103
CML145	P63cOHC181-3-2-1-4-#-2-B-B-B-B-#-#	Late	White	Dent	9.1	0.87	130	97
CML146	AC8563MH35-3-1-B-2-1-B-B-1-B-B-#	Late	White	Dent	9.1	1.08	79	–
CML147	P63c2HC53-1-1-B-B-B-9-B-B-#	Late	White	Dent	10.8	1.01	109	76
CML148	C23QMH19-1-1-B-1-2-B-B-B-B-#	Late	White	Flint	8.6	0.92	87	102
CML149	C24QMH159-2-2-2-B-2-B-B-B-#-B	Late	White	Dent	9.5	0.83	100	96
CML150	C24QMH169-2-1-B-3-1-B-B-3-B-#-#-B	Late	White	Dent	8.4	0.99	90	–
CML151	S8662Q-1-4-4-1-B-#	Late	White	Flint	9.4	1.00	102	111
CML152	S8662Q-1-4-4-5-B-#	Late	White	Flint	9.4	1.00	102	111
CML153	S8662Q-28-4-B-B-B-#	Late	White	Flint	12.1	0.89	108	113
CML154	[EV8762-SR]-17-1-B-B-#	Late	White	Flint	9.5	0.85	108	130
CML155	P62c3HC163-2-1-3#-1-1-1-1-B-1-B-B-#	Late	White	Flint	9.9	0.94	103	116
CML156	P62c3HC163-3-1-3-1-B-1-3-B-3-1-1-B-#	Late	White	Flint	9.0	0.89	120	112
CML157	P62c1HC24-5-3-2-1-B-2-1-1-B-#	Late	White	Flint	8.7	0.87	101	127
CML158	[EV8762-SR]-2-1-B-1-B-#	Late	White	Flint	9.2	0.71	135	90
CML159	P63c2HC5-1-3-1-B-2-1-1-B-#	Late	White	Dent	8.4	1.00	131	102
CML160	P62c6HC6-2-1-1-B-2-1-B-#	Late	White	Flint	10.6	0.97	123	136
CML161	C25Qc18MH520-1-1#-1-2#-5-3-B-1-B-B-B-B-#	Late	Yellow	Flint	11.2	0.82	109	100
CML162	C25Qc1HC18-8-1-2-B-B-2-B-B-B	Late	Yellow	Flint	10.5	1.08	82	101
CML163	C26QMH31-2-2-#-2-2-1-B-1-B-B-#	Late	Yellow	Dent	11.1	0.78	97	115
CML164	P65cHC193-2-7-2#-1-B-1-2-1-B-3-B-1-B-B	Late	Yellow	Flint	9.0	0.89	112	96
CML165	P66c1HC144-3-1-1-B-B-1-B-B-#	Late	Yellow	Dent	11.1	0.83	109	101
CML166	P66c1HC215-4-1-2-B-B-2-B-B-B	Late	Yellow	Dent	10.5	0.88	113	90
CML167	C25QSINT-37-3-2-B-B	Late	Yellow	Flint	11.1	0.88	84	101

CML168	C26QSINT-31-1-2-2-B-B	Late	Yellow	Dent	11.5	0.90	121	110
CML169	C260c22MH7-1-1-1-1-B-B	Late	Yellow	Dent	9.5	0.88	124	126
CML170	C26Qc22MH9-3-1-5-1-B-B	Late	Yellow	Dent	9.6	0.97	90	100
CML171	C25QS4B-MH13-5-B-1-1-2-B-1-B-B-B	Late	Yellow	Flint	10.9	0.90	112	–
CML172	C25QS4B-MH35-2-B-1-1-2-B-4-B-B-B-B	Late	White	Flint	10.8	0.85	–	126
CML173	P68c1HC180-1-3-1-1-B-2-B-B	Late	White	Dent	8.3	0.83	–	–
CML174	P68c1HC249-1-4-4-2-B-B	Late	White	Dent	9.1	1.01	–	–
CML175	P68cOHC77-2-3-7-B-2-3-1-B-1-B	Late	White	Dent	8.6	0.92	–	–
CML176	(P63-12-2-1/P67-5-1-1)-1-2-B-B	Late	White	Flint	7.4	1.05	–	–
CML177	C32QMH84-2-2-1-1-B-B	Late	White	Dent	10.3	0.81	–	–
CML178	C32QMH12-3-1-B-1-B-B	Late	White	Dent	10.0	0.84	–	–
CML179	C32QMH85-2-1-B-2-B-B	Late	White	Dent	10.2	0.88	–	–
CML180	(C32Q/EV8444SRBC4)#-B-#-B-B-21-2-B-B	Inter.	White	Dent	9.2	0.95	–	–
CML181	UWO417-B-2-1-1-B-B	Late	White	Dent	8.8	0.94	–	–
CML182	WOMTA1-B-1-1-1-B-B	Late	White	Dent	10.4	0.86	–	–
CML183	C32QMH36-3-2-2-1-1-B-B	Late	White	Dent	12.4	0.90	–	–
CML184	C32QMH30-2-2-B-1-B-B	Late	White	Dent	12.2	0.80	–	–
CML185	P68C1HC221-3-3-2-3-B-B-B	Late	White	Dent	11.0	0.83	–	–
CML186	P67c2HC26-1-2-1-B-B	Late	White	Flint	8.2	1.00	–	–
CML187	C33QMH2-1-1-2-B-B	Late	Yellow	Flint	10.6	0.88	–	–
CML188	C33QMH2-1-2-2-B-B	Late	Yellow	Flint	8.3	0.92	–	–
CML189	C34QMH17-2-1-1-B	Inter.	Yellow	Dent	10.4	1.04	–	–
CML190	C34QMH103-1-1-2-B	Late	Yellow	Dent	12.6	0.83	–	–
CML191	C34QMH146-1-1-4-B	Late	Yellow	Flint	9.4	1.01	–	–
CML192	C34QMH174-2-1-2-B-B	Late	Yellow	Dent	9.9	0.81	–	–
CML193	CYO162-B-1-1-B	Late	Yellow	Dent	9.3	0.88	–	–
CML194	UYO11-3-2-1-B	Late	Yellow	Dent	8.4	0.88	–	–

[a] Values represent a percent comparison between the yield of each line × tester cross and the mean yield of all lines × tester (thus the yield of the cross CML 140 × Tester 1 was 95% of the mean yield for the crosses of all lines × Tester 1).

[b] Tester 1 = CML144; Tester 2 = single cross CML 146 × CML 150.

Source: CIMMYT 1992.

V. DEVELOPMENT OF HIGH QUALITY PROTEIN CORN

The discovery of new genes or g ene combinations that bring about drastic alterations in plant or kernel characteristics to achieve well defined goals in corn breeding has always fascinated corn breeders. Whenever such new mutants are discovered or identified, attempts are made to transfer them promptly into desired genetic backgrounds. The genetic manipulation of such genes, even when only a single gene or a simple genetic system is involved, seems to be an easy task, yet the achievement of desired goals has often been slow, difficult, and frustrating. Invariably, introduction of such genes brings about other problems which are more difficult to solve. This is particularly true with most endosperm and high quality protein mutants.

A. Early Breeding Efforts

Breeding efforts to develop HQPC have been underway since the early 1960s. The opportune discovery of such HQPC mutants became known at a time when protein-calorie malnutrition and protein gap were issues of great concern in many national programs as well as in several international organizations. Both the developed and developing countries viewed this development with great interest. Breeding programs were initiated to improve the protein quality of corn grain to help improve the nutritional status of malnourished individuals in the developing countries who depend solely on corn to meet their protein and calorie needs. This seemed to be the most logical approach as it would require no change in their food habits.

The early breeding efforts of HQPC pertain to the period between in 1960s and 1970s. The transfer of the biochemical effects of the opaque-2 and floury-2 mutants into more promising genetic backgrounds was attempted worldwide by public and private breeders. The transfer was facilitated by simple and straightforward backcross schemes. The soft chalky phenotype, characteristic of the opaque-2 and floury-2 kernels, aided tremendously to serve as a marker in identifying opaque-2 and floury-2 kernels in the segregating generations. Many breeding programs were thus able to conduct HQPC breeding programs without any pressing need for a sophisticated biochemical laboratory. Initially both opaque-2 and floury-2 genes were extensively used. Attempts also were made on a limited scale to combine opaque-2 and floury-2 to obtain translucent kernels. Another mutant, opaque-7 described earlier, was also discovered during this period but it never found its use beyond experimental investigations. The use of floury-2 gene declined and was essentially discarded as it did not confer any real advantage over opaque-2. The lysine content of floury-2 was intermediate between those of opaque-2 and normal corn,[3] a lower than expected nutritional value detected in swine feeding trials,[86,87] lower test weight of mutant seeds,[88] and high variability among genotypes for seed quality traits.[88,89] As a consequence, more reliance was placed on opaque-2 gene in most active programs around the world as it was simple to transfer because of its true genetic behavior as expected on Mendelian principles.

The conversion programs received major emphasis for almost one decade. Normal elite inbreds entering into specific hybrid combinations and OPVs were converted to opaque-2. A vast volume of opaque-2 corm germplasm was generated from this effort. All converted genotypes possessed standard soft kernel phenotype. Using converted opaque-2 lines, single, 3-way, and double cross hybrids were formed. Extensive yield evaluations were done comparing performance of opaque-2 genotypes with their normal counterparts. By early 1970, the HQPC was ready for commercial exploitation. Some countries, such as Brazil, Columbia, and India among others, made an all out effort to push these materials for commercial production. For some time, these countries and the U.S. showed an upward trend in the production of opaque-2 corn. Production of opaque-2 corn was also underway in some other countries such as Yugoslavia, Russia, and Hungary, but no exact statistics ever became available. By mid-1970, though opaque-2 production was still underway in the above-mentioned countries, the problems confronting opaque-2 corn became quite obvious. The production and interest in growing this specialty corn started declining gradually because of such

problems. A similar experience was observed in breed programs which led to a reduced effort in breeding. Some programs with scarce research resources almost abandoned their activities. Experience gained by breeders developing opaque-2 corn and acceptability studies undertaken in some countries led to the identification of major problems which are described and discussed in the next section.

B. Key Problems Affecting Opaque-2 Corn

Most of the usable HQPC types exhibit similar problems, though much of the experience has been accumulated on opaque-2 corn. These problems have acted as the major stumbling block in the acceptance of such corn by producers, consumers, and the industrial processors. The problems are complex, inter-related, and vary in magnitude and importance depending upon corn usage and various operations relating to corn production practices. A reference to some of these problems was made at a high lysine corn conference that took place at Purdue University in 1966. Several issues and breeding implications of using high lysine genes were emphasized. Later, these problems were further elucidated and reviewed by several research workers.[32,33,35,38,40–42,90]

1. Reduced Grain Yield

Much of the information on yield performance of HQPC is based on the opaque-2 mutation. Standard soft opaque-2 cultivars generally yield lower than their normal counterparts because of lighter and less dense kernels.[34–38,91–98] The yield comparisons have involved both open-pollinated varieties and hybrids. Glover and Mertz[31] have reviewed several studies on yield comparisons, and it would seem that yield responses of different cultivars differ with the genetic background of the material but it would be fair to generalize a reduction of 10 to 15% of opaques compared with their normal counterparts. Yields of a few cultivars that were not significantly different from their normal counterparts have been reported.[34,36,99] Miscovic et al.[100] reported opaque-2 hybrids yielding 86.8 to 94.8% of normal hybrids of the same maturity group.

The reduced yield of opaque-2 cultivars resulted from early cessation of dry matter accumulation in the grain almost 7 to 10 days earlier than the normal corn cultivars.[97] Variations in this period may, however, be encountered, which may then reflect yield differences of different opaque-2 corn genotypes. Most endosperm mutants and all known HQPC mutants affect dry matter accumulation adversely; some, however, affect more drastically than others. In the case of HQPC mutants, it is likely that nitrogen (N) sink capacity may be related to corn productivity.[101,102] It is hypothesized that genetic control of zein and glutelin synthesis may play an important role in promoting the growth and development of normal corn kernels. While both zein and glutelins appear to function as an N sink in the kernel, zein appears to be the most effective sink in this regard because (1) it occurs in greater quantity and (2) its synthesis can be manipulated by N fertilization and genetic means.[101] Also, positive correlations occur between zein content, kernel weight, and grain yield[102] so selection of hybrids showing a large functional N sink might provide a way to increase grain and protein yields per hectare. It is, therefore, important to consider interrelationships between N sink and the carbohydrate sink in corn and other cereals.

2. Unacceptable Kernel Phenotype

All HQPC mutants, including opaque-2, have a common feature in possessing soft, chalky, and dull kernel phenotype. If fed to animals, it does not pose any serious problem. Soft appearance of opaque-2 kernels is not an obstacle in countries having floury endosperm corn, as is the case in the Andean region. However, in many parts of the world when corn is used for human consumption or involves special milling and industrial processing, the soft endosperm of opaque is viewed with concern and is not easily acceptable. This is particularly true in many third world countries where hard flints or dents are generally preferred. It is probable that soft kernel phenotype is caused by

loose packing of starch granules in the endosperm, presence of air spaces, a different protein distribution, and an increased amorphous protein matrix. It has also been hypothesized that endosperm development in corn is a canalized process.[103–105] The canalization hypothesis, though developed for o_7, can be extended to opaque-2. It is agreed that canalization is responsible for observed reduction in the frequency of opaque kernels in populations heterozygous for the mutant allele. It is assumed that the opaque-2 mutant reduces the development activity well below a common threshold.[106] Few genotypes produced by polygene combinations will cross the threshold and modify opaque-2 to a normal phenotype.

3. Greater Ear Rot Incidence

The opaque-2 versions are known to be more vulnerable to ear rot causing organisms.[107–113] Increased incidence of ear rots could be attributed to use of unadapted o_2 donor stocks in initial recoveries, particularly in subtropical and tropical areas. Pericarp splitting, greater vulnerability of soft opaque endosperm, and slower drying resulted from one or a combination of causes. At CIMMYT, when a large number of normal pools and populations were converted to opaque-2, kernel splitting followed by rotting was observed on several opaque-2 genotypes. It seems to be controlled by a simple system but it is highly modified by environment. On some ears a clearcut Mendelian segregation can be observed, while in others this tendency is more heavily concentrated toward the tip of the ear. Genotypic differences also have been observed for their susceptibility to ear rot organisms. A considerable variation may sometimes be observed within the same material.

4. Greater Damage by Stored Grain Insect Pests

HQPC genotypes are more heavily attacked by grain weevils and other stored grain pests because of soft chalky physical structure of the grain.[108,113,114] In many developing countries the infestation may take place in the field itself as corn is left in the field for varying periods following maturation. The greater vulnerability of high quality protein corn genotypes perhaps is independent of high lysine because other soft floury genotypes (floury-1, opaque-1) are more heavily damaged by insect pests.

5. Increased Moisture Content

The opaques tend to dry slower than normals following the physiological maturity of the grain, requiring more days from planting to harvest.[34,39,97,115–117] Some genotypes may, however, exhibit negligible or no difference in moisture content at harvest. It has also been observed that opaque-2 genotypes retained a higher moisture during development than normal, but do not necessarily differ in moisture content at maturity.[118] The black layer, which serves as an indication of physiological maturity, may be seen somewhat earlier but generally coincides with the normals.[35] A difference of a few days may not pose a problem except in those countries or regions that have the danger of experiencing cooler temperatures and early frost during the dry down period. The greater moisture content in opaques over normals, when it occurs, could be attributed to several causes, including thicker pericarp,[119] poor permeability, lack of enough compression against the pericarp thus leaving it somewhat thicker, and finally endosperm structure and composition may also affect dry down ability. Presence of more hydrophilic compounds and more potash (K) may affect dry down ability and thus account for higher moisture content of opaque genotypes at harvest.

6. Poor Germination

In tropical and subtropical environments no marked differences have been observed or reported with respect to seedling growth or germination percentage. In colder regions where temperatures at the time of planting are lower, some opaque genotypes may show slower seedling growth and also poorer germination.[113,115,119–121] It is probable that high prolamine content in corn may be

advantageous for rapid stand establishment. It has been shown that zein is more rapidly mobilized than other proteins during germination, thus accounting for opaque-2 kernels germinating somewhat slower than normal ones.[122]

7. Greater Kernel Breakage

This is not a serious problem in the developing countries as corn is harvested by hand. In the developed world, corn is harvested by machines. Because of the soft physical structure of the opaque-2 grains, such cultivars are more badly damaged than their normal counterparts with respect to cracked and broken kernels.[34,123–125] Varying losses occur depending upon the incidence of such broken or cracked kernels.

C. Breeding Soft Opaque-2 Cultivars

Soft opaque-2 cultivars, whether OPVs or hybrids, can be easily bred by introducing opaque-2 gene into varieties or into lines involved in a particular hybrid combination. To obtain opaque-2 conversions of normal cultivars, a standard backcross program is generally used. The opaque-2 segregates may be recovered from every backcross generation by selfing or intermating, and then used to cross to the recurrent parent to make the next backcross. The conversion process can be accelerated by recovering o_2 segregates after every two successive backcrosses. Continuous back-crossing without advancing generation is also possible, provided backcrossed seed from those plants heterozygous for o_2 allele are identified through a testcross to a homozygous opaque-2 tester. The characteristic phenotype associated with homozygous opaque-2 kernels has tremendously aided in conducting a successful conversion program. Using straightforward backcross procedures, several promising varieties and composites were converted to opaque-2.[38,126] Several good inbred lines involved in hybrid combinations were converted through this breeding procedure and are listed in a manual published by Illinois Foundation Seeds, Inc.[127] In addition to the backcross conversion program, other procedures have been used to develop source opaque-2 populations.

- Introduction of o_2 allele into SSSS (Super Stiff Stalk Synthetic), backcrossed two times followed by random mating and selection of o_2 kernels to form $SSSSo_2$.[128] Syn DOo_2 was developed in a similar manner.
- Crossing several inbreds to an o_2 donor source, advancing by selfing and intercrossing S1s using selected o_2 kernels has been done at the University of Illinois to develop Syn. Ao_2 and Syn. Bo_2.[128]
- Compositing maize varieties from tropical and subtropical origin with simultaneous incorporation of the o_2 allele; Temp. HA o_2 and Temp. HB o_2 were developed through this procedure at Purdue University.
- Intercrossing opaque-2 converted varieties to develop broadbased opaque-2 composites as was done at CIMMYT to develop Comp. K, Comp. J., Comp. 1, and CIMMYT opaque-2 composite.
- Crossing o_2 inbreeds of different origins to develop broadbased o_2 synthetics.[68]

The opaque-2 germplasm so generated by the above methodologies may be used for direct release to farmers, for source o_2 populations for further improvement, and to extract inbred lines for subsequent use in hybrid combinations.

The development of soft opaque-2 hybrids in countries interested in hybrids was mostly done by first converting normal lines through a backcross program, and then putting those lines into a particular hybrid combination. The hybrids released in the U.S., Brazil, Colombia, Yugoslavia, South Africa, and in other countries were mostly opaque-2 analogs of normal hybrids. Very little

or no effort was spent on extracting lines from opaque-2 converted varieties or source populations for hybrid development.

The introduction of o_2 allele into floury-1 backgrounds gets complicated because of similar phenotype associated with both mutants. Some procedures were described by Scheuch & Francis.[129] Using laboratory ninhydrin test, the conversion of floury-1 genotype to opaque-2 can be greatly facilitated. The ninhydrin test permits selection of o_2 kernels in segregating generation, using nondestructive sampling.

D. Breeding Approaches for Developing Hard Endosperm HQPC

The problems confronting HQPC, particularly opaque-2 corn, brought frustration and decline in research interest in HQPC breeding programs around the world. By mid-1970 it was clear that development of opaque-2 OPV_s and hybrids through a straightforward conversion program would not provide acceptable results. While experience on opaque-2 corn was being accumulated, several institutions such as CIMMYT, Purdue University, University of Missouri, and the South African program were exploring other possible approaches which could be used in the development of HQPC. This section will discuss several approaches, including merits and demerits of each approach along with experiences of several research workers around the world. Some of the principal approaches explored so far are discussed below.

1. Search for New Mutants

Ever since the discovery of opaque-2 and floury-2 mutants, the search for new and better mutants has continued. Some new mutants were found, but unfortunately most of these mutants were inferior to opaque-2 and had no practical use in the HQPC breeding program. They also did not offer any advantage in kernel phenotype as they were also soft. Problems of seed lethality and maintenance were additional problems encountered in opaque-6 and floury-3. Attempts have also been made to screen corn germplasm for protein and lysine content in the corn grain.[130–134] Variation has been observed for both protein content and quality with some strains registering lysine values very similar to some of the high lysine mutants. Unfortunately none of the high lysine strains identified through this screening have been used in any practical HQPC breeding program. CIMMYT scientists also did germplasm screening in the late 1960s for high lysine, and identified some families having lysine levels in the high range. Subsequent examination and studies revealed that kernels of several of these families were artifact because of small and shrunken kernels. In summarizing, it may be said that nothing better than opaque-2 has been found. Thus, opaque-2 continues to be the leading mutant for developing HQPC germplasm.

2. Altering Germ-Endosperm Ratio

Altering germ endosperm ratio to favor selection of larger germ size will have the dual advantage of increasing both protein content and its quality.[41,135–137] The germ has twice as much protein as endosperm and has protein quality even slightly better than opaque-2 because the germ contains only small amounts of prolamines. The albumins, globulins, and glutelins, all rich in lysine, constitute roughly 92 to 94% of the total protein in the germ. Variation in germ size exists among and within materials and can be exploited to increase protein quality up to a certain point without adversely affecting other traits with negative correlated response. Though levels of protein quality can be elevated, it will not be easy to attain lysine levels approaching those of opaque-2 corn. Increased germ size will have the disadvantage of contributing to poor shelf life of corn and may be an obstacle to acceptance in those countries which throw away the germ before making various food preparations. Increasing germ size, besides improving the protein quality, will have the indirect effect of increased oil concentration,

resulting in an energy-rich corn kernel and adding to better organolaptic properties for certain food preparations of Africa.

3. Recurrent Selection to Exploit Variation for High Lysine in Normal Corn Breeding Populations

Genetic variation exists among and within materials for protein quality traits. This can be exploited using intrapopulation cyclic recurrent selection schemes to boost the level of lysine in whole corn grain. While difficulties may be encountered in separating genetic effects from environmental effects, this approach does have a practical appeal in improving protein quality through genetic manipulation without altering the physical characteristics of the grain, a problem commonly encountered when high quality protein mutants are used. The agronomic characteristics of the cultivars can be maintained or improved while improving quality protein traits, provided cyclic selection is not limited to this particular trait. Only a few research workers have tried to explore this approach.[41,138–140] Zuber and Helm[139] conducted four cycles of phenotypic recurrent selection in three breeding populations: Midland, Logan County Composite, and Reid Yellow Dent. Encouraging results for improved lysine content after three cycles of selection were obtained. The results after the fourth cycle of selection have, however, revealed only modest gains in lysine and protein concentration. Yield and seed quality were affected adversely, and yield of protein or lysine per hectare failed to increase. Experiences of workers at CIMMYT and Purdue University also have been somewhat disappointing. At CIMMYT, work was initiated on two tropical populations in the early 1970s but was dropped after completing only a few cycles because of narrow genetic variation in lysine and tryptophan, which was further complicated by the protein content. Despite disappointing results, this approach also suffers from other drawbacks:

1. Need for precise analysis and well equipped biochemical laboratories
2. Narrow genetic variation in lysine or tryptophan values, which in turn will result in only a small gain per cycle
3. Difficulty in transferring this trait to other improved populations; at least in the developing countries
4. Environmental variation in protein and lysine content complicated the selection process
5. Lack of assurance that improved protein quality will show superior biological assay

At least in recent years no one has tried to use this approach for improving quality protein traits.

4. Double Mutant Combinations

All known high lysine mutants when tried alone exhibit soft chalky phenotype and encounter other problems presented earlier. Double mutant combinations involving high lysine mutants themselves or in combination with some endosperm mutants have been attempted to alter at least unacceptable properties of standard soft opaques. Two combinations which deserve special mention are opaque-2 floury-2 ($o2o_2fl_2fl_2$) and sugary-2 opaque-2 (su_2o_2). Both combinations have been produced, and experiences of those involved in exploiting these combinations are discussed below.

a. Double Mutant Opaque-2 Floury-2 Combination

This combination generated interest as it produced translucent kernels.[91] At CIMMYT, in earlier stages, attempts were made to produce double mutant combinations in a wide array of genetic backgrounds to improve upon protein content and kernel appearance. Unfortunately, CIMMYT breeders did not encounter translucent shiny kernels resulting from the interaction of these mutant genes. Other breeding programs have also met with limited success. It is likely that translucent

kernels occur in rare genetic backgrounds, but overall experience worldwide did not favor continuation of this project.

b. Sugary Opaque-2 Double Mutant Combination

Extensive work has been done at Purdue University to exploit interaction of opaque-2 and sugary-2 mutants to develop phenotypically appealing HQPC. Work was also initiated at CIMMYT on the advice of Purdue University scientists. A modest effort was initiated in mid-1970 to develop this mutant combination. Much of the experience gained and available to date has come from the work of these two institutions. The translucent kernel phenotype of su_2o_2 corn has been reported by several research workers.[42,141–144] In addition, the su_2o_2 segregates are either equal to or better than their counterpart o_2 kernels in protein quality. Some general conclusions, based on work conducted at Purdue University, CIMMYT, and some other research institutions, can be made on this double mutant combination.

1. The su_2o_2 segregates manifest varied kernel phenotypic expression in different genetic backgrounds. The degree of modification ranges from partial modification to complete modification. In addition, the soft inner core was visible even in vitreous su_2o_2 segregates.
2. The su_2o_2 segregates, in general, exhibited an intensification of endosperm color.
3. In dent backgrounds, the su_2o_2 segregates showed dentation without presence of soft cap.
4. The su_2o_2 segregates were smaller compared to o_2 counterpart. They were lower in 100 kernel weight though higher in kernel density compared with their soft o_2 counterpart. Considerable variation was observed between and within materials.
5. Homozygous su_2o_2 ears tended to shell more easily.
6. Rows on su_2o_2 ears lacked compactness. The open spaces were observable between and within rows.
7. The protein content and quality were not very different, though in some backgrounds the lysine values in protein were slightly higher.
8. The su_2o_2 ears generally had more number of kernel rows.
9. The incidence of ear rot was reduced but was still inferior to normals.
10. The su_2o_2 kernels have higher oil content because of higher percent oil values in both germ and endosperm tissue and slightly higher germ to endosperm ratio — but not as a result of increased germ weight.[145,146]
11. The su_2o_2 kernels can withstand breakage and mechanical stress better than soft o_2 kernels and are even superior to normals.
12. In su_2o_2, the glutelin synthesis is increased, which may thus contribute to higher yield of lysine per endosperm than their mutant o_2 kernels.
13. Germination and seed vigor characteristics of su_2o_2 may be similar to or inferior to o_2.[147] The modified phenotypic appearance of su_2o_2 kernels does not contribute to better field emergence.

The combination aroused considerable interest primarily because of translucent kernel appearance, and because of slightly better protein quality and thus improved biological and nutritional value. The greatest drawback of this combination is its reduced grain yield. On the average, the yield reduction may be as much as 20%. Apparently it seems that the two mutants act in an additive manner to reduce the accumulation of dry matter in the grain. Kernel phenotype, though the kernels are translucent yet their appearance is somewhat dull, would require additional selection work to add uniformity to kernel appearance. Also, in regards to milling, it may have promise for dry milling but would require adjustment in the milling procedures to accommodate this specialty corn.[125] Yield disadvantage of this combination will be a major limiting factor in the acceptance of this double mutant corn. Consequently the efforts at CIMMYT were abandoned a decade ago as other approaches described in later sections appeared more promising in producing HQPC with

respectable yield and greater acceptance. The effort at Purdue University is also at a bare minimum, limited to only experimental investigations.

5. Selection for Multiple Aleurone

Increasing the number of aleurone layers has been suggested as a possibility of increasing the amylose quality of normal corn. Multiple cell layers were seen in a floury race Croico.[148] This characteristic may be influenced by the genetic background of the material, but genetic studies have revealed that it may be conditioned either by a single, partially dominant gene[148,149] or by two partially dominant genes with genetic modifiers influencing it.

6. Combined Interaction of o_2 and Genetic Modifiers to Develop QPM Germplasm

This approach exploits the use of the o_2 gene in combination with the genetic modifiers to overcome major defects in developing agronomically desirable HQPC cultivars with modified texture of the grain. Recognizing the magnitude and the importance of the problems, a desired association of the opaque-2 gene with genetic modifiers can be sought. Much of the earlier work using this approach has emphasized improvement of kernel phenotype, which was rated as the major hurdle in the acceptance of high lysine corn — at least in the developing countries. Modified, mosaic, or variegated patterns were observed by the breeders in the earlier days during the conversion program, but were consciously discarded for fear of contamination and for lack of proper understanding. Thus opaque-2 phenotype on one hand aided as a marker, while on the other hand it forced breeders to eliminate partially modified kernels whose useful role was later discovered. Of all the approaches that have been tried worldwide, this seems to be the only viable breeding approach to develop QPM with acceptable yield and agronomic characteristics. Several aspects of genetic modifiers affecting kernel modification and their secondary associations with others were described and discussed earlier. This section will cover various aspects of breeding hard endosperm opaque-2 materials. Since much of the work has been done at CIMMYT exploiting this approach, the experience of CIMMYT scientists will be discussed somewhat in greater detail. In general, conscious concerted efforts have been lacking at other institutions. Some universities in the U.S. and some national maize programs have once again revived their interest in QPM with the use of CIMMYT corn germplasm.

The development of hard endosperm opaques at CIMMYT dates back to 1969 to 1970. Though major effort at that time was on developing soft opaque-2 cultivars though a conversion program strategy and by developing new opaque-2 composites, an exploratory work on modified opaques had already begun.[150] The initial interest was on improving kernel phenotype. As work progressed, however, there were indications that the whole array of complexly inter-related problems affecting soft opaques could be remedied by the modifying gene complex. By this time, additional reports on modified opaques had appeared.[45] In the early 1970s, a concerted effort was made to select modified opaque-2 ears from different opaque-2 converted populations that were available at that time. Selection of the best modified kernels from each ear was done and planted ear to row. The materials were carried forward either by selfing or by plant to plant crosses within the family followed by selection of best modified ears; the best selected kernels from each ear were advanced to the next generation. By mid-1970, we were getting good indications and enough confidence that this approach would work. The development and improvement of soft opaques was de-emphasized from this point onwards. Relevant practical experience was also gained by this time, indicating that concomitant selection against other undesirable attributes could also be done. It had also become obvious that a few other considerations should be kept in mind while exploiting such modifiers. Regarding kernel modification, a regular modification would be easy to manipulate during the selection process. A continuing pressure also should be kept at all stages to discard

dull modifiers, which appear translucent but lack attractiveness and appeal. Improvement in level of kernel modification should receive emphasis at all the stages during the breeding cycle. Between ear selection should be complimented with within ear selection for most modified kernels. The effect of maternal influence should be minimized by using appropriate selection schemes during the development and the improvement stages. Protein quality should be monitored, and endosperm analysis is preferred over the whole kernel analysis because the modification involves changes in the endosperm. As level of kernel modification approaches the normal phenotype, a switch-over to whole lysine analysis could be made at this time. From the past experience of working with so many materials, the CIMMYT breeders and biochemists would strongly favor endosperm analysis for at least several initial cycles. It is important that in the segregating generations, the modified opaque-2 kernels should be selected with extra care. Temptation to select completely modified kernels should be avoided. To accelerate progress, selection should be practiced in homozygous opaque-2 backgrounds. The final product ought to be uniform and stable for kernel appearance for better market acceptance. Additional selection criteria should be considered in the field to attain faster progress. Eliminate ears with loose kernel rows and open spaces between rows, providing indication that such genotypes do not reach their full potential with respect to dry matter accumulation in the grain. In an indirect way this helps to select for better kernel weight in modified textured hard endosperm genotypes. Early harvesting should be practiced to facilitate selection of faster drying genotypes to match the moisture content of normal genotypes. Ears exhibiting popping or split pericarp tendency, as well as slower drying ability should be eliminated to reduce ear rot incidence. Stability of kernel modification is important, but such selection should preferably wait until later generations when good kernel modification may have been achieved. Strong selection pressure for stability in earlier stages when modifications is not good would unnecessarily eliminate good germplasm.

The development of hard endosperm opaque-2 maize germplasm passed through several distinct phases before CIMMYT scientists could gain enough confidence to use this approach on a large scale. Initial efforts were started by Asnani[150] with assistance of Johnson and Lonnquist. Various categories of modified kernels were sorted out from partially modified ears selected from opaque-2 composites and the backcross derived o_2 populations. Protein content and quality was monitored during the selection process, but it had become apparent in the early stages that selection for modified phenotype kernels adversely affected the quality protein traits. Increased laboratory support was thus built up, and from this point onwards the breeders and biochemists worked together in an integrated interdisciplinary approach to develop the QPM germplasm. Germplasm development and improvement activities were diversified to provide an even spread of sample analysis work in the biochemical laboratory. The important sequential phases through which the QPM program has progressed will be described.

a. *Building Up Resource Genetic Base*

The development of hard endosperm opaques was initiated by CIMMYT from the existing germplasm, which at that time possessed soft endosperm texture. Initial efforts were directed at encountering variations for partially modified ears in opaque-2 materials during the conversion process, improvement of existing opaque-2 composites, formation of opaque-2 population crosses, and during seed increase of opaque-2 materials for international testing trials. Since a wide array of germplasm was under the conversion program, a few hundred partially modified ears were separated from several different genetic backgrounds. Several opaque-2 populations were also identified that showed a higher frequency of modified phenotype opaque-2 ears. Populations that substantially contributed to the development of this germplasm included population cross Ver. 181-Ant. Gpo.2 × Ven. 1 opaco-2, Composite K, Thai opaque-2 composite, and Composite I. Selected partially modified ears were shelled individually, followed by selection of the best modified kernels from each ear. The selected kernels were planted on an ear to row basis. The best plants were selfed, and at harvest good modified ears were selected for further work. Both between and within ear

selection was emphasized, coupled with laboratory analyses to build up the raw QPM germplasm that provided the future base for the QPM work. The initial effort was not only difficult, but quite tedious and at times very frustrating.

b. Development of QPM Donor Stocks

The initial germplasm selected in phase 1 was used to form opaque-2 donor stocks that were later used in the conversion program as nonrecurrent parents. In developing donors, two principal approaches were used. Intrapopulation selection was practiced in some populations exhibiting a relatively higher frequency of modified o_2 ears. Five such populations in which either full-sib or half-sib selection was practiced are Composite K, Ver. 181-Ant. Gpo. 2 × Ven. 1 opaco$_2$, Thai opaque-2 composite, PD(MS)6H.E.o_2, and Composite 1. The second approach involved recombination of partially modified Opaque-2 families selected from different genetic backgrounds. The white and yellow families were recombined separately to form White Hard Endosperm opaque-2 and Yellow Hard Endosperm opaque-2 composites. Additional recombination with selection helped to improve their performance for subsequent use as donor stocks. In addition to serving as donor stocks, both materials appeared promising for improvement through intrapopulation improvement schemes.

c. Development of QPM Germplasm

The development of QPM germplasm in the CIMMYT corn program was accomplished using the following approaches.

Recurrent Selection in QPM Germplasm — Some of the QPM donor stocks described in the preceeding section seemed to be good candidates for the recurrent selection program. Full-sib recurrent selection using international progeny testing data was practiced in these populations for at least two to five cycles. In addition, site specific and across site experimental varieties were developed by recombining ten full sib families based on international progeny trial data. These improved populations and experimental varieties were made available to national programs and to other private and public institutions on request.

Backcross-Cum-Recurrent Selection Program — Once fairly acceptable QPM donor stocks were available, an attempt was made to convert potentially useful tropical and subtropical germplasm to hard endosperm opaque-2. The backcross procedure had to be modified as it involved two genetic systems governed by the opaque-2 gene and the genetic modifiers. Traditional or standard backcross programs will incorporate the opaque-2 gene but the genotypes will fail to have desired kernel modification. A combination of backcross and recurrent selection procedure was used in achieving this goal.[41,42,57] The procedure combines several merits:

1. Use of improved version of recurrent parent every cycle
2. Quality of kernel modification determines the next backcross
3. Facilitates carrying forward families in homozygous o_2 background
4. Prevents dilution of already accumulated modifiers
5. Permits selection of other traits in addition to modifiers

The procedure does slow down the recovery of recurrent parent, but it does have the merit that one does not start over following each backcross. The homozygous fraction with increased frequency of genetic modifiers can be used for yield evaluation and other purposes when needed. It is important to emphasize that during the conversion process, utmost attention must be given to all the plant and seed characteristics that will upgrade the performance of QPM materials for yield, kernel appearance, ear rot resistance, and drying ability. A number of CIMMYT populations have been converted to QPM using this method. The yield performance of QPM and their normal counterpart is given in Table 4.4 Though yield differences between normal and QPM vary, there are some genotypes where the differences are negligible.

TABLE 4.4
Comparison of Normal Materials and Their QPM Versions for Yield and Other Agronomic Traits

Material	Yield in t/ha		QPM and % of normal	Ear height (cm)		Days 50% silking		Ear rots (%)	
	Normal	QPM		Normal	QPM	Normal	QPM	Normal	QPM
Tuxpeño 1	6.19	6.15	99.4	111	110	61	60	2.2	5.4
Mix. 1 Col. Gpo. 1 × Eto	6.12	5.68	92.9	107	105	60	58	2.9	4.5
Mezcla Amarilla	5.43	5.24	96.4	103	106	58	58	2.9	5.7
Amarillo Dentado	5.35	5.23	97.7	125	110	61	57	2.5	3.2
Tuxpeño Caribe	6.39	5.90	92.3	117	115	61	58	2.5	4.2
Ant. Rep. Dominicana	5.35	5.08	94.9	100	112	56	57	2.3	3.7
La Posta	6.47	5.90	91.2	131	122	62	59	3.1	4.0

Formation and Improvement of QPM Pools — The principal objective in forming these pools was to accumulate genetic modifiers from as many diverse sources as possible. A number of tropical and subtropical QPM gene pools were formed in at least two ways. The first approach was to recombine modified phenotype opaque-2 families originating from several source materials into a QPM pool with a specific climatic adaptation followed by selection and accumulation of more desirable modifiers in the subsequent cycles. The second approach involved crossing normal genotypes with similar climatic adaptation to as many different hard endosperm opaque-2 donor stocks as possible. The resulting F1 crosses were genetically recombined in a half-sib recombination block in isolation. At harvest, F2 ears exhibiting the least segregation for soft o_2 segregates were selected. Modified o_2 segregates were sorted out from each ear separately. Genetic mixing continued in a homozygous o_2 background in the following cycles with major emphasis on the accumulation of modifiers while maintaining protein quality. Detailed information on the handling of QPM gene pools has been described and discussed in earlier publications.[41,42,57] The names and description of some of the important tropical and subtropical pools is given in Table 4.2.

The evaluation of different cycles of selection has shown improvement in plant height, ear height, maturity, ear rot incidence, and endosperm hardness score. The performance data on cycles of selection of some of the pools is presented in Table 4.5. Yield improvement was observed in some pools but not in others. The latest cycles in general tended to be earlier, shorter in plant and ear height, have reduced ear rot incidence, and to have lower ear modification scores. The mean endosperm hardness score of kernels in different cycles has progressively improved over cycles (Table 4.6). The improvement in kernel modification is evident from lower values with every successive cycle of selection. The upper and lower limit values also showed an improvement trend from the earliest to the latest cycles (Table 4.7). Protein content and quality did not shift much during successive cycles of selections. These gene pools could be used as donor stocks, elite fraction as a new population for improvement and introgressing superior families into corresponding population, and extraction of QPM inbred lines.

Shift in Germplasm Management Strategy — The breeding approaches described in the preceding sections facilitated the development of a wide array of QPM germplasm with modified kernel characteristics and superior agronomic attributes adapted to tropical and subtropical environments. At this point it became more important to design a new management strategy that would facilitate handling of QPM germplasm more efficiently. Two major changes were introduced and implemented; the first involved the merging of genetically similar QPM materials having phenotypic resemblance to reduce the volume, and the second pertained to working in homozygous opaque-2 backgrounds to further accelerate progress. This route in germplasm merging process was taken

TABLE 4.5
Evaluation of Cycles of Selection of Four QPM Gene Pools

QPM gene Pool	Cycle	Yield (t/ha)	Plant height (cm)	Days to silk	Moisture (%)	Ear aspect
Pool 33 QPM	CO	4.11	212	58	22.2	4.2
	C12	4.45	192	55	22.2	1.9
	L.S.D. (.05)	0.19	6.7	0.7	1.1	0.3
Pool 34 QPM	CO	4.36	213	58	22.3	4.3
	C12	4.68	187	55	21.9	2.2
	L.S.D. (.05)	0.25	7.3	0.6	1.1	0.3
Pool 32 QPM	CO	4.56	204	55	20.1	3.5
	C12	4.83	192	55	21.8	2.2
	L.S.D. (.05)	0.36	7.4	0.8	2.0	0.3
Pool 23 QPM	CO	3.34	203	56	21.0	4.1
	C11	3.46	198	55	21.1	2.4
	L.S.D. (.05)	0.21	11.3	1.1	1.3	0.3

TABLE 4.6
Mean Endosperm Modification Scores of Different Cycles of Selection in QPM Pools

	Kernel modification[a]			
QPM pool	CO	C4	C9	LSD (.05)
Pool 23 QPM	3.7	2.8	2.3	0.20
Pool 24 QPM	3.6	2.6	2.3	0.17
Pool 25 QPM	3.2	2.6	2.3	0.16
Pool 26 QPM	3.2	2.6	2.4	0.43
Pool 32 QPM	3.7	2.8	2.3	0.20
Pool 33 QPM	3.1	2.6	2.1	0.26
Pool 34 QPM	3.0	2.5	2.2	0.20

Note: LSD — Least Square Difference

[a] Rating scale 1 to 5:1 — completely vitreous; 5 — completely soft.

realizing that most materials resulting from the conversion program had received enough backcrosses, and that perhaps little would be gained by pursuing additional backcrosses. On the other hand it was felt that the pace of progress could be accelerated by subjecting such materials to recurrent selection programs. Also, since much of the QPM germplasm has been developed at CIMMYT using only a few environments, some of these materials would benefit tremendously by multilocation international progeny testing trials. This would permit further improvement in yield, stability, and general adaptation over varied environments. The merging process also helped to accomplish formation of major germplasm types that are widely needed in the developing countries. In making these decisions we relied heavily on our past experience in the international maize testing program. The entire QPM germplasm was thus systematically consolidated into 10 QPM populations and 13 QPM gene pools, which are listed in Tables 4.1 and 4.2. The number, name, and some phenotypic features of these QPM materials are also listed in those tables.

Upon completion of the reorganization process, all the newly formed QPM gene pools and populations, which were homozygous for the opaque-2 gene, were subjected to intrapopulation improvement schemes. The QPM gene pools were handled by a modified half-sib system.[41] The

TABLE 4.7
Mean Kernel Modification, Standard Deviation, Range, and Weinberg Constant in Different Cycles of Selection in Pool 33 QPM (Sample of 20 Ears from Each Cycle)

Cycle of selection	Mean kernel[a] modification	Range	Standard deviation	Weinberg constant
CO	3.65	4.84 to 2.94	1.07	1.60
C4	2.87	3.66 to 2.26	0.92	1.57
C9	2.20	3.48 to 1.24	0.91	1.23

[a] Rating scale 1-5: 1 — completely vitreous; 5 — completely soft.

half-sib system was periodically interrupted for an inbreeding phase to expose and discard deleterious recessives, improve inbreeding stress tolerance, identify superior S1 lines for inbred development and to permit sampling of new lines from the more advanced cycles on a continuous basis.

The QPM populations were improved by modified full-sib family selection using multilocation international progeny testing trials. Each cycle was completed in two years and the improvement process involved four stages:

1. Regeneration of full-sib progenies
2. Evaluation at six test sites internationally
3. Within-family improvement by selfing during the intervening cycles
4. Recombination of the best S1s from selected full-sib families by bulk sibbing among families

In addition to cyclic improvement, narrow based site-specific and across-site experimental varieties were formed for further evaluation in experimental variety trials in the developing countries. A more detailed description of the improvement system can be found in several CIMMYT publications.[42,43,126]

E. QPM Hybrid Development at CIMMYT and in Other Institutions

Up until 1984, the major thrust in CIMMYT's QPM program was on population improvement to develop open-pollinated varieties. The emphasis was shifted to QPM hybrid development in 1985. Several advantages were advocated for QPM hybrids over the open-pollinated varieties including 1) improving yield performance through exploitation of heterosis, 2) facilitate maintaining the seed purity of inbred progenitors with respect to agronomic traits, the genetic modifiers, and the protein quality, 3) reduced dependence on laboratory facilities for monitoring protein quality provided the lines are fixed and kept genetically pure, 4) the hybrids will exhibit more uniformity and stability with respect to kernel modification, and 5) attracting involvement of the private seed industry in QPM effort.

The principal objectives of CIMMYT's QPM hybrid program were to: (1) evaluate combining ability of existing QPM germplasm in various phases of development, (2) develop inbred progenitors and assess their *per se* and combining ability performance, (3) develop single, three-way, and double cross conventional hybrid combinations, (4) evaluate superior hybrid combinations for targeted areas, and (5) announce and make available to both private and public sector inbreds with superior performance.

The combining quality of CIMMYT's tropical and subtropical QPM germplasm was studied and the results published.[151–153] In tropical QPM germplasm, populations 62, 63, and PR7737 exhibited significant positive GCA for yield, and Pool 23 QPM, Pool 25 QPM, Populations 62,

64, and 65 had significant negative GCA for endosperm modification. The negative values for kernel modification are considered desirable for this trait. Based on our results, Populations 62 and 63 among white materials and Population 65 among yellow materials could be used for initiating hybrid work. The results from subtropical diallel indicate that Population 68 QPM, Pool 32 QPM, Population 69 QPM, and Pool 34 QPM hold good potential for QPM hybrid development in the subtropics. For the temperate breeding programs in the U.S. Pool 27 QPM, Pool 29 QPM, and Population 70 QPM could be used as exotic germplasm for introgression into corn-belt HQPC materials and for developing hard endosperm QPM hybrids. In regards to kernel modification, the flint QPM materials were, in general, better combiners. Bjarnason and Vasal[44] and Villegas[154] have described activities of CIMMYT QPM hybrid maize program. Inbred progenitors have been developed from the existing tropical and subtropical QPM germplasm. The lines that survived inbreeding were tested for combining ability and the superior ones put into hybrid combinations. The yield performance of some of the superior hybrids is presented in Tables 4.8A and 4.8B. The better performing QPM hybrids yielded equal to, and in some cases better than, the normal check entries. A number of QPM inbreds were announced by CIMMYT in 1992 and are being made available to national programs and to other private and public research organizations. These lines will help QPM hybrid development efforts in those countries interested in hybrid development.

Hybrid development efforts in HQPC have been underway in some other institutions as well and are still being pursued quite vigorously. Crow's Hybrid Seed Company in the U.S. has high lysine corn hybrids in the market for several years and has maintained continued interest in the development of superior and better hybrids. In the last decade or so, in addition to developing soft high lysine corn hybrids, the company has also placed emphasis on the development of QPM

TABLE 4.8A
Superior Yellow QPM Hybrids Tested across Seven Locations at El Salvador, Guatemala, and Mexico, 1998

Pedigree	Yield (t/ha)	Ear rot (%)	Tryptophan (%)	Ear modification	Silking (days)	Plant height (cm)
CML172xCLQ66061	6.71	2.5	0.104	2.0	54	260
CML161xCML165	6.50	7.7	0.111	1.4	55	232
CML169xCML172	6.30	9.9	0.096	2.4	56	256
95HT74Q	6.08	9.6	0.090	1.6	54	255
CML168xCML172	5.83	4.7	0.102	1.7	55	252
CLQ6503xCLQ6601	5.77	4.4	0.104	2.4	51	241
ACROSS 8765 TLYF	5.08	13.2	0.100	1.9	52	225
Normal hybrid check	5.50	8.8	0.060	2.2	56	241

Local checks: CB-HS-86M23, CML297XCL02410, H-104, HA-50, HET-9122.

hybrids with modified endosperm texture. Several QPM materials from CIMMYT have been used as source germplasm in conjunction with their own materials in developing inbred progenitors and hybrid combinations. It is likely that they will start commercializing new QPM hybrids on the market before too long. Glover[144] has reported that the commercial high-lysine (o_2) cultivars marketed by Crow's Hybrid Company have been improved and that better hybrids yield 89 to 95% of normal check hybrids. The best adapted hybrids will yield within 5% of normal check hybrids.

In South Africa, the University of Natal has pursued vigorously the development of high lysine corn hybrids. In the beginning, the emphasis was on developing standard soft opaque-2 high lysine hybrids which resulted in the release of a white high-lysine o_2 hybrid 'HL1' in 1979. In recent years, Gever[58,155] described that the program has placed major emphasis on the development of modified opaque-2 corn hybrids with selection strategies based on several criteria including relative

TABLE 4.8B
Superior Subtropical White QPM Hybrids Tested across Six Locations at Mexico, Zimbabwe, and Uganda, 1998

Pedigree	Yield (t/ha)	Ear rot (%)	Tryptophan (%)	Ear modification	Silking (days)	Plant Height (cm)
CML176xCML142	8.22	4.8	0.082	1.7	80	268
CML176xCML146	7.98	9.9	0.080	1.9	79	260
QS7705	7.88	5.1	0.082	1.8	79	248
CML186xCML149	7.40	6.0	0.090	1.5	76	248
CML173xCML142	7.85	4.3	0.082	2.0	75	245
CML176xCML175	7.81	4.2	0.087	2.2	76	243
Normal hybrid check	7.36	5.1	0.064	2.2	78	252

Local checks: H-358, A-7545, H-312.

kernel mass (RKM), visual screening and selection of modified opaque-2 kernels, biochemical analysis to monitor quality characteristics, making kernel density and hardness measurements, studying electrophoretic patterns of selected samples, and performing agronomic and nutritional evaluation. In 1982, 'HL2', the first South African yellow high lysine hybrid was released commercially. It is a modified single cross and is competitive in yield and agronomic performance to the best normal commercial hybrids. It has good acceptability, a kernel phenotype nearly indistinguishable from the normal, and extremely good resistance to ear-rot diseases.

The QPM hybrid development effort at Texas A&M is perhaps more recent. Buckholt and Rooney[155,156] have reported progress on the development of QPM hybrids for the U.S. Several QPM materials from CIMMYT were testcrossed using their own tester parents. They identified several populations, 66, 68, 69, and temperate × tropical that were good combiners and belonged to the Lancaster group. Pool 29, also a good combiner, was identified as belonging to Reid Yellow Dent heterotic group. Two other populations, 63 and 65, combined very well with both testers. An inbred line development program was carried out by inbreeding and selections within the CIMMYT's QPM populations and by converting standard U.S. inbreds to QPM. In the conversion program, the soft opaque-2 versions of normal U.S. inbreds were used. The program at Texas A&M has made excellent progress in developing lines and hybrids. They may soon propose release of a white and yellow QPM hybrids which have competitive yield and agronomic performance.

Brazil has revived interest in QPM. In addition to open-pollinated QPM cultivars, the program is placing a new research thrust on the development of yellow QPM hybrids.[157] The program is producing both inbred and non-inbred progenitors to produce both conventional and non-conventional QPM hybrids. Several yellow QPM single cross hybrids have been evaluated and some of them will be used to produce three-ways and double crosses in the future. The program also intends to incorporate new techniques of molecular biology in inbred line development.

F. Progress in Overcoming Problems

It seems from past experience that the QPM germplasm, developed using combined interaction of the opaque-2 gene and the modifiers, is the only viable strategy. Research at CIMMYT and elsewhere has amply demonstrated that some of the problems plaguing soft HQPC can be solved satisfactorily. This section discusses progress achieved to date with respect to some of the most serious problems.

1. Improvement in Kernel Phenotype

A dramatic improvement in kernel phenotype has been achieved in several QPM materials. The frequency of soft ears has declined continuously with successive cycles of improvement (Figure 4.1). The kernel modification score, rated on a scale of 1 to 5, has shown continuous improvement

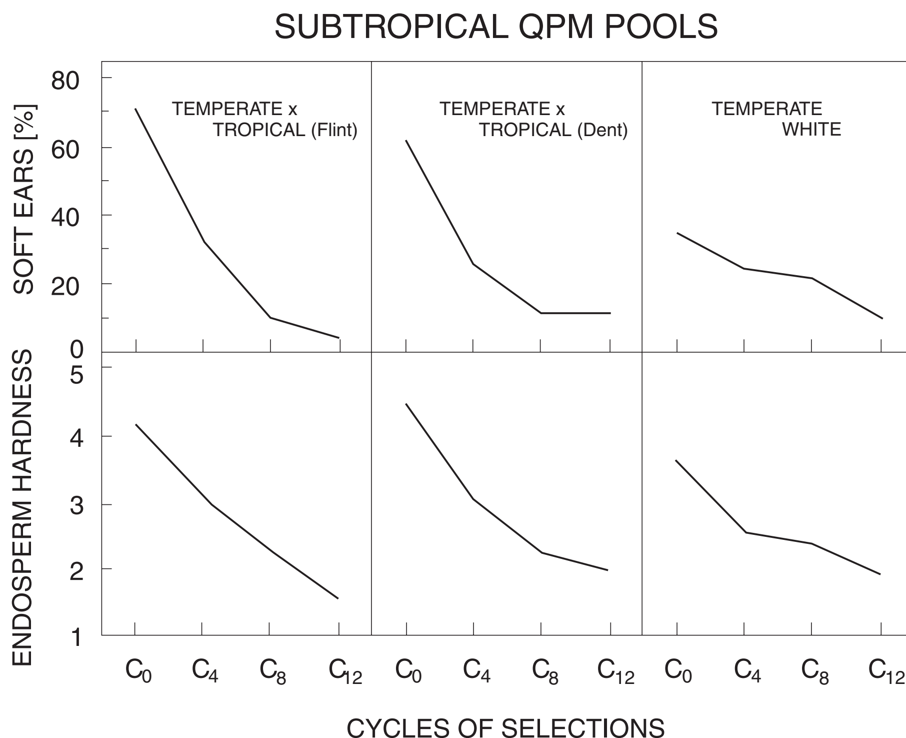


FIGURE 4.1 Percentage of soft ears and endosperm hardness scores in different cycles of selection of 3 subtropical QPM gene pools.

as judged by the lower ratings in the most recent cycles. The standard deviation and the Weinberg constant indicated that the most recent cycles were less variable (Table 4.7). Both the upper and the lower limit values showed a decreasing trend from the original to the latest cycle of selection. A change in the frequency of different modified classes also occurred during the selection process (Figure 4.2). The frequency of classes with a rating scale of 4 and 5 has continued to decline while that of classes 1, 2, and 3 has continued to increase; the latest cycles invariably show the highest frequency in these three classes. It may be interesting to emphasize that even when modification at the ear level has markedly improved, many more cycles of selection are needed to stabilize kernel modification and to reduce the variation within the ear to a minimum level.

2. Yield Advances

Many corn breeders around the world have abandoned HQPC breeding work primarily because of reduced grain yield. A number of approaches have been used singly or in combination with others to reduce the yield gap between normals and QPM. Figure 4.3 shows yield advances over the years and Table 4.4 shows that several QPM materials yielded as well as the normal check entries. Comparison between QPM and comparable normal backgrounds also revealed similar performances[159] (Table 4.9). Good yielding hybrids have also been reported by Gevers (Table 4.10)

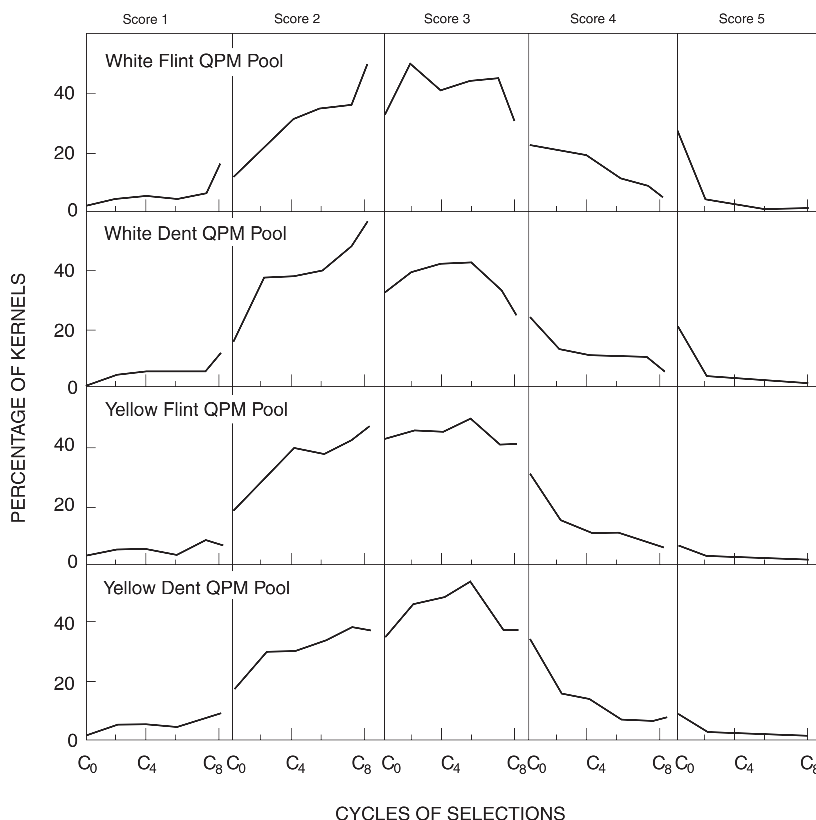


FIGURE 4.2 Relative frequency of different modified kernel classes in different cycles of selection of four tropical QPM gene pools. Rating scale 1 to 5: score 1, — completely vitreous; score 5, — completely soft.

from South Africa and Buckholt[156] from Texas A&M. This could be attributed to improved dry matter accumulation in the grain in QPM germplasm.

3. Reduced Ear Rot Incidence

Substantial progress has been made in improving ear rot resistance in QPM germplasm. Several factors including improved kernel phenotype, better drying, reduced influence of temperate germplasm, reduced frequency of alleles causing pericarp splitting, and selection for ear rot resistance both under natural and artificial inoculation conditions have contributed to the reduced incidence of ear rot. However, international trial data show that QPM materials still show slightly higher ear rot incidence compared with normals (Figure 4.4).

4. Better Drying Ability

Several factors may have contributed to better grain dry down ability, including early harvesting and improved dry matter accumulation. Currently available QPM materials show no difference in the moisture content from normals in the same genetic background.

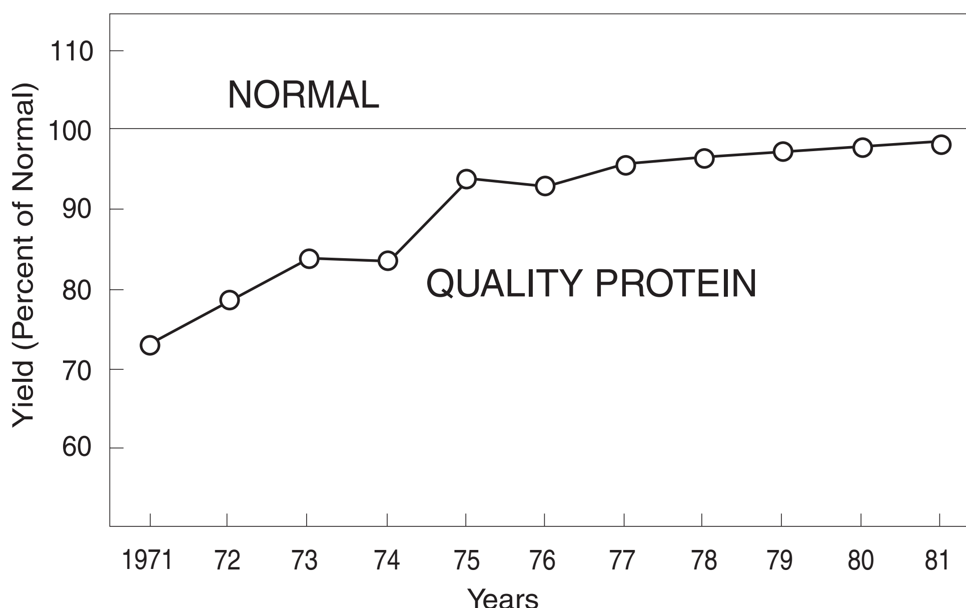


FIGURE 4.3 Grain yield performance of QPM corn expressed as a percentage of normal maize check in different years across all test locations.

TABLE 4.9
Across Location, Performance of Normal Materials and Corresponding Tropical QPM Germplasm, 1987

Material	Grain yield (kg ha-1)		QPM as percentage of normal
	Normal	QPM	
Pool 23	5405	5330	98.6
Pool 24	5706	5457	95.6
Tropical High-oil	5733	5170	90.2
Population 62	5347	5484	102.6
Population 65	5255	5369	102.2
Population 63	5705	6236	109.3

Source: Bjarnason and Short, 1988.

5. Improved Resistance to Stored Grain Pests

Improved kernel phenotype has indirectly helped to reduce incidence of stored grain pests. Limited data available suggest that QPM materials are less affected by stored grain pests as compared to the soft opaques.

6. Maintenance of Protein Quality

During the course of germplasm development and improvement, protein quality was continuously monitored by simple, rapid, and efficient methods developed by Villegas and his co-workers.[160] Table 4.11 shows protein quality of several QPM tropical and subtropical populations. Most of these materials have good protein content and good lysine and tryptophan values. The protein quality also has been maintained in different cycles in pools and populations (Table 4.12). The

TABLE 4.10
Modified Opaque-2 Hybrids Grown at 12 Locations in South Africa (1987–88)

Hybrid	Mean yield % of mean	Mean density (g/cc)
PNR (N)	117	1.22
HL-8	114	1.24
HL Com.	111	1.22
XHL	111	1.25
N.C. (N)	108	1.21
HL-2	101	1.22

Source: Gevers, H. O., University of Natal, South Africa, 1989.

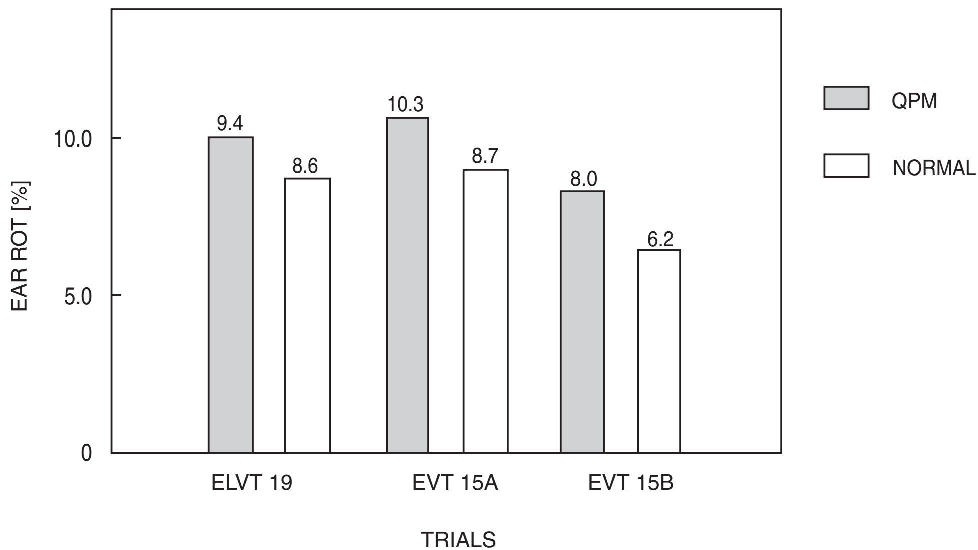


FIGURE 4.4 Mean performance of QPM entries and normal checks across locations in different trials conducted during 1980 for ear rot (%).

protein fractions of several QPM populations and of three versions of Tuxpeño are given in Tables 4.13 and 4.14. In general, all modified opaque-2 versions continued to show lower levels of fraction II.

VI. TYPES OF CULTIVARS AVAILABLE

Both open-pollinated and hybrid HQPC cultivars can be developed for commercial production in the farmer's field. Among the HQPC cultivars, one can exercise several options depending upon the gene or gene combinations contributing to high protein quality. The experience gained over the years by the corn breeders has dictated the use of the opaque-2 gene almost exclusively for development of this type of specialty corn. Other genes do not offer any additional advantage over that of the opaque-2. The discussion in this section will thus be limited to cultivars developed

TABLE 4.11
Protein, Tryptophan, and Lysine in the Whole Grain of QPM Populations

QPM Population	Protein (%)	Tryptophan in protein (%)	Lysine in protein (%)	Quality index
Population 61	9.2	0.98	4.2	3.8
Population 62	9.9	0.92	3.9	4.4
Population 63	9.1	0.97	4.3	4.3
Population 64	9.6	1.00	3.8	4.3
Population 65	9.2	0.96	4.2	4.4
Population 66	9.3	1.01	4.3	4.3
Population 67	9.9	1.04	3.9	4.8
Population 68	9.5	1.01	4.0	4.3
Population 69	10.0	0.98	4.2	4.4
Population 70	9.3	1.10	4.3	4.7

TABLE 4.12
Protein, Tryptophan and Lysine in Different Cycles of Selection of Four QPM Materials (Endosperm Analysis)

Material	Cycle	Protein (%)	Tryptophan in protein (%)	Lysine in protein (%)	Quality index
Yellow flint QPM	C0	8.0	0.78	2.90	3.2
	C6	8.0	0.75	2.90	3.2
PD (MS)6 QPM	C0	8.5	0.78	2.50	2.8
	C4	8.8	0.78	2.60	3.0
Temperature × Tropical QPM	C0	8.0	0.66	3.20	3.2
	C8	8.0	0.68	2.90	3.2
White O_2 Back Up Pool	C0	8.5	0.85	3.40	3.1
	C6	7.8	0.74	2.90	3.3

TABLE 4.13
Protein Fractionation of Some QPM Materials (Endosperm Samples)

Material	Fraction I (Albumins + globulins)	Fraction II (Zein)	Fraction III (Zein-like)	Fraction IV (Glutelin-like)	Fraction V (Glutelins)
Population 61	13.6	10.8	17.6	19.8	38.2
Population 63	14.6	10.9	18.4	21.2	34.7
Population 66	13.8	13.4	20.4	17.6	34.7
Pool 17 QPM	12.8	10.8	23.7	19.4	33.4
Across 8141	15.0	11.7	19.7	19.3	34.2
Poza Rica 8140	14.7	13.4	18.6	19.0	34.2
Pichilingue 8039	15.0	13.1	19.6	18.6	33.6
Guanacaste 7940	13.9	16.0	21.7	17.5	30.8
Across 8140	16.0	11.6	19.8	18.8	34.0
Tuxpeño-1 (Normal)	6.2	39.2	19.7	13.6	22.4
Tuxpeño o_2 (Soft)	20.6	8.1	10.7	18.5	42.5

TABLE 4.14
Protein Fractionation of Normal, Soft Opaque and Hard Endosperm Opaque-2 Versions of Tuxpeño-1

Fraction		Tuxpeño normal	Tuxpeño soft opaque-2	Tuxpeño H.E.o_2
I	(Albumins + globulins)	6.2	20.6	15.5
II	(Zein)	39.2	8.1	10.4
III	(Zein-like)	19.7	10.7	16.2
IV	(Glutelin-like)	13.6	18.5	21.4
V	(Glutelins)	22.4	42.5	36.6

through the use of the opaque-2 gene alone or in combination with genetic modifiers to develop hard textured endosperm opaques.

The choice of OPVs vs. hybrids will vary from one country to another and is guided by several factors including strength of national program, trained manpower, and existence of infrastructure of seed production by both public and private industry. In generals, hybrids are more popular in the developed world. In the developing countries, both hybrids and OPVs are grown with some countries (Argentina, Brazil, El Salvador, Egypt, Turkey, Thailand, China, and Zimbabwe) growing mostly hybrids. Equally important is the issue of growing either soft opaques or modified endosperm textured HQPC. In countries where corn is grown for feed purposes, soft kernel phenotype does not present problem. Soft kernel phenotype is also not an obstacle in countries having preference for floury corn, as is the case with several Andean regional countries. In many developing countries where corn is principally used for human consumption, hard flints and dents are generally preferred. The need for soft or hard endosperm will thus be dictated by corn use for food, feed, or industrial purposes.

Historically, HQPC with standard phenotype was generally available and released. It is only recently that the modified phenotype opaque-2 cultivars have started gaining more popularity. CIMMYT's success in developing modified endosperm opaques has generated renewed interest and revived HQPC breeding activities in some countries. It may be highlighted that interested countries in the developing world have relied heavily on the QPM breeding effort conducted at CIMMYT. These countries have used QPM populations, gene pools, experimental varieties, and synthetics developed at CIMMYT. Even in the U.S., some universities pursuing research on HQPC have used CIMMYT materials as source germplasm in the new strategy to emphasize breeding of modified phenotype opaque-2 lines and hybrids. Of particular interest is the work underway at the University of Purdue, University of Illinois, Urbana, Texas A&M, and Crow's Hybrid Seed Company. Interesting and challenging work on modified opaques has been undertaken by Brazil, China, and the Republic of South Africa. Other countries are pushing more towards usage rather than developing HQPC, thus depending on CIMMYT for germplasm assistance.

A vast volume of materials developed by CIMMYT was described earlier. In addition to the basic source germplasm with different adaptations, maturities, grain color, and texture, CIMMYT has developed several hundred experimental varieties synthesized by recombination of ten superior families selected on the basis of international progeny testing trials. Beginning in 1985 as described earlier, QPM hybrid development was emphasized, thus resulting in the formation of synthetics, inbred lines, and hybrids. Much of this germplasm has been evaluated in either standard experimental variety trials (EVTs) or in special hybrid trials. Recently QPM inbred lines, which carry CML numbers *140 to 194* have been announced. The announced inbreds belong to both tropical and subtropical adaptations. *In recent years testing of QPM hybrids has been intensified resulting in identification of superior hybrid combinations for several developing countries.*

The HQPC cultivars released in different countries are listed in Table 4.15. It includes mostly OPVs, hybrids, and inbreds. It should be emphasized that many of the materials released in the early or mid-1970s are no longer in production. Such is the case with two Colombian opaque-2 hybrids (H208, H255) and several Agroceres hybrids (Ag. 502 and Ag. 503) in Brazil.

Excellent research conducted by Crow's Hybrid Seed Company and by the Republic of South Africa is particularly noteworthy. Both of these organizations have pursued HQPC breeding work with persistent enthusiasm and have made many varietal releases. Crow's Hybrid Seed Company has developed high lysine corn hybrids for grain and for silage. At least two hybrids for silage and a dozen hybrids for grain are being marketed in a somewhat restricted area. They will soon have modified opaque-2 hybrids on the market.

The Republic of South Africa had earlier released *three* hybrids — HL1 (White), HL2, (Yellow), and later HL8, which has hard endosperm, possesses good yield potential, and has tolerance to diseases. Recently several white and yellow QPM hybrids have been released. Six white QPM hybrids (HL19, HL25, HL23, QS7701, QS7705, GH132-28) and four yellow QPM hybrids (QS7600, QS7602, QS7610, QS7608) have been commercialized by Quality Seeds. In addition a yellow QPM hybrid, NS9100 has been released by National Seed. Agronomic data indicates that most of the QPM hybrids are competitive in yield, plant and grain characteristics, standability, and disease resistance.

The interest of Chinese scientists in QPM is exceedingly important because of the swine industry. They have made an all-out effort to release OPVs and hybrids. Tuxpeño 102 is an OPV directly released from CIMMYT source population Tuxpeño QPM. In addition to two soft endosperm hybrids, Zhong Tan #206 and Zhong Tan #209, they have developed a hybrid Tan (Shandong SC) #203 using lines extracted from CIMMYT population 70 (Templado Amarillo Dentado QPM). The hybrid is at least 10% better in yield over Zhong Tan #205 and #209. Chinese researchers are making extensive use of QPM germplasm developed by CIMMYT. They have improved photoperiod sensitivity of two CIMMYT QPM, Pools 33 and 34, under long day conditions. The improved populations have been renamed as C. Pop 13 and C. Pop 14. As mentioned earlier, China holds a good potential for dissemination and promotion of QPM hybrids. The ongoing efforts in different provincial academies have received renewed interest all across the country. The Chinese government also has shown great commitment to promote QPM efforts in the whole country. In August 1999, a QPM hybrid, Zhongdan 9409, was released in a special ceremony attended by Vice Minister of Agriculture, CAAS President, Vice-President, Director General of CIMMYT, Mr. Sasakawa and his accompanying team/group from Japan, Dr. Norman Borlaugh, and several other CIMMYT and CAAS researchers. Field days are held each year. There is a new wave of excitement and enthusiasm to promote and spread QPM. In Guizou, southern province, QPM intervention is being used to alleviate poverty and to improve the well-being of farmers. In addition to Zhongdan 9409, a few other hybrids, Zhongdan 3850 and Zhongdan 3710, are in advanced stages of testing and release.

In Brazil, emphasis on QPM research has continued on variety and hybrid development. Two QPM varieties, BR451 and BR473, have been commercialized. These varieties were developed by the breeding program of the National Maize and Sorghum Research Center (CNPMS-EMBRAPA). The variety BR451 is white and was released in 1988. It has been successfully used as a substitute for wheat because of its white color. The QPM variety BR473 is yellow and was released in 1994. The genetic background of BR451 is CIMMYT QPM Population 64. It has white dent kernels with a good level of endosperm modification. The yellow QPM variety BR473 is an early maturity yellow synthetic originating from four flint and two dent QPM lines. A QPM hybrid BR212 was also released in 1997.

The Ghana Sasakawa Global 2000 project is promoting QPM in Ghana. The release of QPM variety Obatanpa in Ghana in 1992 has made significant advances in extending the spread of this variety in farmer's fields. This variety currently accounts for 61% seed sales of total improved variety maize seed sales in the country. It is estimated that in 1998 1500 tons of certified seed was sold for commercial grain production. The QPM hybrid development effort has received increas-

TABLE 4.15
High Quality Protein Corn Cultivars Available in Different Countries

Country	Variety name	Source germplasm	Type
Argentina	RAE-10	Opaco semi-dentado	OPV
	Tuxpeño opaque	Tuxpeño opaque-2 (Pop.37)	OPV
Bolivia	Tuxpeño O_2	Tuxpeño opaque-2 (Pop.37)	OPV
	Chuquisaca 7741	Templado Amarillo QPM (Pop.41)	OPV
	IBD-5	Subtropical germplasm	OPV
Brazil	BR451	Blanco Dentado-2 GPM (Pop.64)	OPV
	BR473	Six line yellow synthetic	OPV
	BR2121	—	Hybrid
China	Tuxpeño 102	Tuxpeño QPM	OPV
	Hybrid 201	$Mo_{17}(O_2)$ × Italian hybrid 308	Hybrid
	Line 079	Yellow H.E.o_2 (Pop.39)	Inbred
	Hybrid 203	SC201 × Line 079	Hybrid
	Zhongdan-2	—	Hybrid
	Zhung Tan #206	—	Hybrid
	Zhong Tan #207	—	Hybrid
	Zhongdan 9409	—	Hybrid
	Zhongdan 3710	—	Hybrid
	Qian 2609	—	Hybrid
Ecuador	INIAP-528	AC. 8363 (Pop.63)	OPV
Guatemala	Nutricta	Tuxpeño QPM	OPV
Ghana	Obatanpa	Blanco Dentado-2 QPM (Pop.63)	OPV
	Dedaba	3-way crosses	Hybrid
	Mamaba	3-way crosses	Hybrid
	CIDA-ba	3-way crosses	Hybrid
Guinea	Obatanpa	Blanco Dentado-2 QPM (Pop.64)	OPV
Honduras	Nutricta	Tuxpeño QPM	OPV
India	Shakti-1	Modified opaque-2	OPV
Mali	Obatanpa	Blanco Dentado-2 QPM (Pop.64)	OPV
Mexico	H365	(CML142xCML150)xCML186	Hybrid
	H469	CML146xCML186	Hybrid
	V537	Poza Rica 8763	OPV
	V538	Across 8762	OPV
	H522	CML164xCML161	Hybrid
	H523	CML165xCML161	Hybrid
Paraguay	Nutri-Guarani V-241	Yellow Dent QPM	OPV
Peru	Opaco Huascaran	Composite J	OPV
Senegal	Obregon 7740	White H.E.o_2 (Pop.40)	OPV
	Temperate White QPM	Temperate White QPM	OPV
South Africa	HL1	—	Hybrid
	HL2	(B0385YxB0395)xDO 940Y	Hybrid
	HL8	(B0385YxB0395Y)xK0135Y	Hybrid
	HL19	White	Hybrid
	HL25	White	Hybrid
	HL23	White	Hybrid
	QS7701	White	Hybrid
	QS7705	White	Hybrid
	GH132-28	White	Hybrid
	QS7600	Yellow	Hybrid
	QS7610	Yellow	Hybrid

continued

TABLE 4.15 (CONTINUED)
High Quality Protein Corn Cultivars Available in Different Countries

Country	Variety name	Source germplasm	Type
	QS7602	Yellow	Hybrid
	QS7608	Yellow	Hybrid
	NS9100	Yellow	Hybrid
Venezuela	AC. 7740 Funit-2	White H.E.o_2	OPV
Vietnam	Population 63	Blanco Dentado-1 QPM (Pop.63)	OPV

ingly greater emphasis in recent years. Several QPM hybrids have been developed and evaluated. In 1997, three QPM hybrids were released. The hybrids are GH-110-5, GH-132-28, and GH-2328-88. Mass utilization of QPM variety in Ghana is exemplary. Several potential commercial channels for QPM utilization have been identified, including infant and institutional feeding programs and animal producers, especially poultry and piggery farms.

The successful introduction of Obatanpa has generated interest in the neighboring countries of Africa, including Benin, Togo, Burkina Faso, Nigeria, Gambia, Sierra Leone, Mali, Ethiopia, Tanzania, Congo, Mozambique, Ivory Coast, and Senegal. Two countries in the region, namely Mali and Guinea, have officially released Obatanpa. Interest in Obatanpa and QPM hybrids has also grown in eastern and southern African countries. A 3-way QPM hybrid, Dadaba, is under evaluation in Tanzania and southern Africa. It is hoped that Quality Seed Company will take up seed production of this hybrid for distribution throughout southern and eastern Africa.

The Maize Directorate in India has released a QPM variety Shakti-1 in 1998. It yields well and has acceptable modified opaque-2 phenotype. At present only a limited seed quantity is produced and distributed.

The government of Mexico has shown renewed initiative, enthusiasm, and commitment in promoting and disseminating QPM varieties and hybrids. Extensive trials with QPM hybrids have been conducted all across the country and some have shown potential for commercial release as judged by their performance in these national trials. The data revealed that several QPM hybrids were better or equal in performance to the best available commercial normal-endosperm hybrids from public and private institutions. This has resulted in a cooperative agreement between the government of Mexico and CIMMYT to promote QPM on a national scale. Initially promotion activities will be restricted to high yielding environments with good chances of adoption and to the extent possible in areas with established swine and grain processing industries. An ambitious seed increase program has been undertaken to produce enough seed of QPM varieties and hybrids to plant 2.4 million ha in the next 2–3 years. INIFAP is launching an all-out effort to register 21 QPM hybrids and 4 open-pollinated varieties for different regions of Mexico. QPM hybrids selected include white and yellow types as well as single, 3-way, and varietal hybrids. A 3-way QPM hybrid CML142*CML150/CML186 and a single cross QPM hybrid CML176*186 have shown outstanding performance. Many hybrids under consideration for release involve CMLs144, 141, 142, 176, 186, 150, 159, 145, 158, and 149. A number of hybrids have given yields touching 12–14 tons/ha.

The optimism is once again gaining strength. In the course of the next few years we may witness exciting developments in the growth of QPM in many developing countries.

VII. NUTRITIONAL AND ECONOMIC BENEFITS OF HQPC

The nutritional and biological superiority of HQPC has been amply demonstrated in rats,[161,162] pigs,[86,163–165] infants and small children,[166–169] and adults.[170–173] Also, the nutritional superiority of HQPC has been summarized in several review articles.[31,32,172–175] Experimental studies on rats have shown that young rats grow faster on opaque-2 corn as compared to their normal counterparts. The

protein efficiency ratio (PER) of opaque-2 corn (2.78) resembles very closely that of Casein (2.88) at comparable levels of protein in the diets. The niacin availability was also better from opaque-2 corn. Similar results were also achieved in pigs with dramatic differences in the growth of animals fed on opaque-2 and normal corn. Maner[165] concluded from his studies that opaque-2 corn can be used as the only dietary source of protein during the finishing, pregestation, and gestation periods of a pig's life cycle without reducing pig performance. It was, however, pointed out that opaque-2 corn was not adequate for baby pigs, growing pigs, or lactating sows and must thus be supplemented with some protein to obtain maximum performance. In the case of poultry or chicks, the opaque-2 can show better gains and feed conversion than normal corn at below optimum protein levels only when supplemented with methionine, for which chicks have a higher requirement. In Guatemala, Bressani[166] showed that opaque-2 corn was 90% of the nutritive value of milk protein in young children. The children in Colombia suffering from a severe protein deficiency disease (Kwashiorkor) were brought back to normal health on a diet containing only opaque-2 corn as the source of protein.[172]

The recent report implies that just like children, the adults also would benefit greatly from QPM because of its higher level of lysine and tryptophan.[178] Graham and co-workers[179] have presented interesting results that babies in the second year of life grow normally when QPM is fed as the only source of protein in the diet.

More recent studies indicate that advantage of QPM in starter and growers pigs diets.[180] Also, the researchers in South Africa have shown that their new HL2 yellow QPM hybrid may be of ruminants or dairy cattle. Benefits in increased milk production could also result when farmers feed o_2 corn silage to dairy cattle.[149]

The opaque-2 corn also can help to reduce the protein supplement if used as a gradient in animal feed. Studies at the University of Nebraska[180] have shown that when Crow's Hi-lysine corn was fed in place of normal corn to all classes of pigs from weaning to finishing, the total level of dietary protein supplement could be reduced by 2%. Researchers in South Africa also have reached similar conclusions. Gevers[155] has indicated that the use of high lysine corn (HLM) in monogastric animal feeds and its direct industrial exploitation could offer greatest immediate rewards. Using HLM in pig feeding trials, it was shown that 22% of fish meal normally used in pig diets could be saved, principally due to the increased lysine content of HLM. Potentially this savings can be converted to considerable economic advantage by the feed manufacturers and on-farm users of HLM.

In CIMMYT's economic program, Lopez-Pereira has studied the role of QPM as an ingredient in the animal feed in Brazil and El Salvador.[182] The results of the study suggest that the use of QPM as a feed ingredient in pig feed could help reduce the costs depending on the QPM-to-regular corn and soya bean-to-regular corn price ratios.

QPM surpasses ordinary corn in biological value and net protein utilization. Although highly digestible, the true digestibility is slightly inferior to normal corn.[183] Bressani[173] has reviewed studies on children and has shown that QPM was the only protein source fed to children recovering from malnutrition. This resulted in increased nitrogen balance and improvement in health condition. He made a suggestion that QPM can be a practical solution to homemade weanling foods.

The QPM offers tremendous benefits both in human and animal nutrition — particularly in monogastric animals. In human nutrition intervention, it may play a very important role in those countries where corn constitutes staple food in the diets of children and adults. In the words of Normal Borlaug,[184] "It is time, I believe, to make a serious effort to put it into commercial use to serve human needs." QPM, as an ingredient in animal feed, may have a far more important role to play. Increasing the use of corn as animal feed globally would provide indirect benefits to humans and would result in greater impact.

VIII. FUTURE OUTLOOK

The past 25 years have witnessed important happenings in the breeding of HQPC and its role both in human and animal nutrition. The early optimism of those who advocated this specialty corn solely on the basis of superior nutritional quality could not be realized because of poor agronomic characteristics of this corn. Nutritionists also added confusion from time to time and there was divergence of opinion on the issue as to the relative importance of calories and proteins in human diets. Evidence has accumulated over time that this corn can offer enormous benefits, provided the agronomic deficiencies can be rectified. Most breeders worked hard but lost the battle in upgrading agronomic performance of this corn to the level of normal corn. While the abandoning of HQPC breeding effort happened worldwide, only three institutions had the courage and continuing interest in pursuing this goal. The institutions are CIMMYT, the South African program at the University of Natal, and Crow's Hybrid Seed Company at Milford, Illinois. The breeding effort at CIMMYT was, however, substantially greater than the other two organizations. Breeders at CIMMYT had explored all possible avenues and breeding approaches before actually embarking on a strategy that produced fruitful results. In the opinion of CIMMYT breeders, the only viable strategy has been the one based on the accumulation of genetic modifiers in conjunction with the opaque-2 gene. A great breakthrough was made by using this strategy, resulting in the development of QPM germplasm that compares in performance to the normal corn in yield and kernel characteristics. The success story at CIMMYT and in South Africa has revived interest once again in several countries. Brazil and China are heavily using CIMMYT germplasm and have made releases recently. They have expanded their effort on hybrid development and have a good chance of placing QPM hybrids on the commercial scene in the next few years. The announcement and release of QPM lines by CIMMYT will help accelerate hybrid breeding efforts in some countries. It may also encourage private seed industry to develop and market QPM hybrids.

The Sasakawa Global-2000 African Agricultural program is placing a major thrust in promoting QPM in sub-Saharan Africa. Currently efforts are concentrated in Ghana. A QPM release has already been made which will cover several thousands of hectares with QPM in this country in the next few years. The efforts will be extended to other African countries as well in the near future.

Thus, evidence is mounting that good QPM can be bred. It is hoped that these new developments will provide excitement and new challenges to corn breeders working in this area. We may also foresee expanding use of molecular genetic tools in developing QPM lines. At least programs at Texas A&M, Brazil, and South Africa are beginning to use these new tools. The o_2 DNA probe has already been introduced into the program to facilitate the development of new o_2 lines. The South African program studies electrophoretic patterns of selected samples as an important criteria. The Brazilian program is planning to incorporate molecular biology tools in an inbred-line development program. Renewed challenge in QPM has prompted interest in establishing how the modification of the endosperm is affected at both biochemical and molecular levels.[185–188] There is already indication that modified o_2 kernels contain increased amounts of gamma-zein protein. It is highly plausible that the increased synthesis of gamma-zein is a consequence of modifier gene activity. At present the mechanism by which an increase in gamma-zein content could result in a vitreous endosperm is unclear. Also, the higher content of this protein does not explain the high lysine content of the grain. Since QPM genotypes maintain high lysine content, this implies that there must be a significant increase in some lysine-rich protein fractions. Better characterization of zein proteins in the future will thus facilitate QPM conversion efforts. It may also become possible to distinguish modified o_2 kernels from normals by RP-HPLC (reversed-phase high performance liquid chromatography) and other sophisticated techniques.

The interest in QPM development efforts has been growing in many countries in recent years. Acceptable performance of QPM germplasm with protein quality as an added bonus should prompt

national governments to replace normal corn for human consumption and as a feed grain in the U.S. and other corn producing countries to reduce the cost of animal protein. Promotion, commercial production, and utilization of QPM should now receive priority; at least in those countries where QPM has given a good performance.

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5 High-Oil Corn Hybrids

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I. INTRODUCTION

The objective of this chapter is to discuss factors involved in the development and use of high-oil corn hybrids. The chapter will discuss data on animal feeding trials, potential market, selection for

increased oil quantity, and programs to develop high-oil corn hybrids. In addition, factors involved in corn oil quality and their potential in high-oil corn hybrids is discussed. The first published research forming the basis of breeding corn with improved protein and oil concentration was by C. G. Hopkins et al. in 1903.[1] Their classical report titled "The structure of the corn kernel and the composition of its different parts" formed the basis for divergent mass selection, started in 1896 to change the chemical composition of the corn kernel relative to protein and oil concentration. Hopkins et al.[1] divided the corn kernel into six major parts: (1) tip cap, (2) hull (pericarp), (3) horny glutinous part (aleurone), (4) horny starchy part (horny endosperm), (5) white starchy endosperm (soft starch), and (6) germ. They estimated 80 to 84% of the total oil is located in the germ, 12% in the aleurone, and 5% in the endosperm. These 1903 estimates agree with a recent summary of data by Watson.[2]

The potential use of corn oil as a food or feed was noted by Smith in 1908.[3]

> The oil of corn has in recent years formed such a wide commercial use that under the present market conditions, it has become, pound for pound, by far the most valuable constituent of the grain, and whereas formerly in the glucose factories and corn mills the germs containing the oil were almost a waste product. There is now an actual demand on the part of these industries for corn which is richer in oil. It is proposed to meet this demand by breeding corn for increase of oil content.

This statement is still applicable today to the development of high-oil corn. The importance of corn oil in margarines, salad oils, cooking oil, and a large number of food products is well known; less well known is the use of high-oil corn to enhance the feeding rations of animals.

II. FEEDING TRIALS

The major projected use of high-oil corn is for animal feed and human food. In the past 30 years, several feeding trials with chickens, swine, sheep, and dairy cattle have all shown advantages from increasing the level of corn oil in the ration.

A. Poultry

Han et al.[4] compared high-oil and normal corn rations for laying hens and broiler chicks. Laying hens receiving a diet of 5.7% oil, 9.5% crude protein, and 14% moisture vs. a normal diet of 3.6% oil, 8.7% crude protein, and 14% moisture experienced no increase in egg production, body weight, feed consumption, and egg yield over the normal diet. Laying hens fed a diet of 17% protein plus high-oil corn, from 23 to 38 weeks of age, had a better egg-to-feed ratio than those fed a normal ration, and egg production and yield tended to increase with the high protein, high oil diet.

A comparison using broiler chicks from 8 to 22 days posthatch and diets of high-oil (6 to 13%) vs. normal corn (4.5%) showed the high-oil diet was superior in weight–gain-to-feed ratio.[4] Additional studies with adult roosters found a high-oil corn diet produced 4.5% more metabolized energy than a normal corn diet.[4] Blood plasma and shank pigmentation were increased in the high-oil diets. Chick feeding trials conducted in Yugoslavia using diets containing 7% high-oil vs. 4% oil corn found the high-oil diet was significantly superior when fed for 56 days after hatch.[5] Chicks fed the normal corn gained 952 g vs. 1,006 g for the high-oil diet. Feed conversion was 2.58 kg feed kg^{-1} gain for the high-oil diet and 2.75 for the normal diet, indicating an increase in feed efficiency for high-oil diet. Additional poultry feeding trials using high oil corn diets with 70 g kg^{-1} or higher high oil corn have shown similar results.[6]

B. Swine

The adage that swine fed diets high in fat produce pork of poor quality, or "soft pork," has been misleading. Several swine feeding trials have shown if the diet fat level is below a certain level,

high quality pork can be produced. A limited unpublished study feeding high-oil corn to swine conducted at Iowa State University in the early 1950s was designed to measure fat quality. Kastelic[7] reported rations containing 7.4% vs. 3.4% corn oil had no effect on fat quality of the pork when measured by iodine number. Nordstrom et al.[8] compared 7% vs. 4% oil diets for finishing swine and found the high-oil diet required about 6% less feed lb^{-1} of gain than the normal diet. An additional study using 15% high-oil corn in the swine ration did produce "soft" or poor quality pork.[8] Swine rations with up to 8% oil in the diet usually do not produce "soft pork," but oil levels above this usually do. The genetic strains of swine could affect this response.

Swine feeding trials by Adams and Jensen[9] using finishing swine and reproducing sows compared three isocaloric diets, 7.5% oil, 3.5% oil, and 3.5% plus refined corn oil (4.0%) to obtain 7.5%. Nonsignificant differences in average daily gains for the three diets were observed, but the high-oil diets had lower feed intakes than normal diet. The average gain-to-feed ratio of the high-oil diets were significantly higher than conventional diet (0.55 and 0.53 for two high-oil vs. 0.50 normal oil diets). High-oil corn diets fed to sows resulted in significantly greater body weight gains and higher fat levels in the colostrum than normal-oil diets. However, litter size, birth weight, and weaning weights were not affected by the high-oil diet.[9] The data indicated swine are able to utilize corn oil in the whole kernel as effectively as processed corn oil added to the ration. These results could allow high-oil corn to be used in formulating swine rations and could eliminate problems associated with adding animal fats or other oils to the ration.[10]

C. Dairy Cattle

Feeding trials with lactating dairy cattle[11] fed diets containing 6% high-oil corn vs. 4% oil corn showed a 12% greater intake of dry matter for the high-oil diet (21.9 vs. 19.6 kg $d.m.^{-1}$).[11] No difference was observed for total milk production of the two rations, but the high-oil ration produced an increase in body weight from 4 to 20 weeks postpartum and the normal ration showed a decrease in body weight during this period.

D. Sheep

Garrigus[12] compared normal and high-oil (7%) rations, high in protein (11 to 12%) for lambs. Lambs on the high-oil, high-protein ration gained 7% more weight, retained more nitrogen, and required 6% less feed lb^{-1} of gain than the normal diet.

Feeding trials conducted over the past 30 years all show an advantage from feeding high-oil corn rations to chickens (8% or more), swine, dairy cattle, and sheep over normal rations. Additional farm feeding trials are needed to confirm the advantages of high-oil corn in animal rations.

III. POTENTIAL USE OF HIGH-OIL CORN

To estimate the potential of high-oil corn, it is possible to use the yearly estimates of feed consumed and to estimate the necessary hectares required to produce this size crop.[13] In 1997 the U.S. harvested 29.8 million hectares (79.5 million acres) of corn that produced 23.7 million metric tons (9.3 billion bushels) of grain.[13] Livestock consumed about 15 m metric tons (5.9 million bushels) or about 63% of the 1997 crop. The five states of Illinois, Indiana, Iowa, Minnesota, and Nebraska harvested about 64% of total area planted to corn in the U.S. These five states produced 14.67 million metric tons of grain (5.8 billion bushels) which is close to the 1997 supply fed to livestock.[14] To plant the 1997 acreage in these five states would take about 15.7 million bags (bag contains 80,000 viable kernels).[15] Value-added traits such as high oil, improved oil quality, better balanced amino acids, and lower phytate level would add considerable value to the hybrid seed and also an additional return to the grower for producing this type of hybrid.

IV. DEVELOPMENT OF HIGH-OIL CORN HYBRIDS

A. INTRODUCTION

High-oil corn hybrids are generally considered to have a kernel oil concentration greater than 6%. Conventional corn hybrids usually range in oil values from 3.5 to 5.0%.[2] The 6% level has some basis in fact, because when this oil level is fed in animal rations usually a significant feed response over a normal ration is observed. For a 55-year period (1917–1972) the average oil content for corn processed at three corn wet-milling plants in the Corn Belt varied from 4.9 to 4.2% oil[16] with no apparent trend over this period. Similar data collected by Watson[2] for normal corn processed by wet-milling plants and grown in four contiguous midwest areas (Iowa, Illinois, Wisconsin, and Indiana) showed a slight decline in oil values from 4.9% in 1954 to 4.3% in 1982.

The corn germ consists of scutellum and embryo, the latter containing the primary axis, plumule, coleoptile, mesocotyl, adventitious and primary roots, and the coleorhiza.[17] The scutellum in normal corn makes up about 10 to 12% of the total kernel dry weight, but the scutellum contains 83 to 85% of the total oil in the kernel. The pericarp tip cap and endosperm contain between 10 and 13% of the total oil in the kernel. The scutellum consists of epithelial, paraenchyma cells, and vascular tissue. Oil synthesis in the developing scutellum tissue deposits oil bodies or spheresomes in the parenchyma cells of the scutellum.[17]

Kernel development proceeds very rapidly after pollination, with the endosperm cells developing at a much more rapid rate than the germ shortly after pollination. By 15 days after pollination (DAP) the endosperm and germ have developed to a size that chemical determinations can be made.[18] In developing normal kernels oil concentration can be detected 15 DAP and continues to increase to about 45 DAP and then remains relatively constant to physiological maturity or black layer.[19] A comparison of the rate of oil accumulation in kernels of a high-oil inbred and a normal inbred showed a higher rate of oil accumulation for the high-oil inbred during the 15 to 45 DAP period. Both inbreds had relatively constant rates of oil accumulation from 45 DAP to black layer.[20] Misevc̈ et al.[21] compared oil accumulation rates in whole kernels of three groups of hybrids with total oil concentrations in the 5, 7, and 9% range between 21 and 56 DAP. Significant rate differences in oil accumulation among the three groups were observed, with the highest accumulation rate for the 9% oil hybrids and lowest for the 5% oil hybrids.

The size of spheresomes has been estimated to range from 1.09 µm for Illinois low-oil (ILO at 0.5% oil); to 1.31 µm in Illinois high-oil (IHO at 18% oil).[22] Oil body size in the endosperm is estimated to be much smaller, <0.1 µm.[23] One inference from these estimates is that higher oil genotypes contain slightly larger oil bodies and have a greater number of oil bodies. Usually high-oil hybrids and other high-oil genotypes have a larger germ size, which is mostly scutellar tissue, so a greater number of oil bodies is possible.

Data in Table 5.1 compare several kernel traits means of two normal (B73 and Mo17) with eight high-oil inbreds for several kernel traits (Lambert, unpublished). As expected, the high-oil inbreds had higher oil levels than the normal inbreds (44 vs. 117 g kg^{-1}, Table 5.1). The high-oil inbreds were significantly higher in protein and lower in starch concentration. There was variation among the high-oil inbreds for these traits. Kernel size as measured by kernel number $volume^{-1}$ and kernel weight show there are more high-oil kernels $volume^{-1}$ than normal kernels (121 vs. 157) and kernel weight is less (212 vs. 265) for the high-oil inbreds. The high-oil UHO inbreds with higher oil concentrations also had smaller kernel size compared with AEC high-oil inbreds. In addition, germ size was larger for the higher oil inbreds (13.4 vs. 22.4) and lower in endosperm size (86.7 vs. 77.6) than normal inbreds (Table 1). The data show high-oil inbreds developed from synthetic varieties (AEC and UHO) that have been selected only for increased oil concentration result in inbreds with increased germ size, reduced endosperm size, and an increase in concentration of oil in the germ.

TABLE 5.1
Means for Several Kernel Traits for Two Normal and Eight High-Oil Inbreds Averaged over Two Years (1993–1994)

				Dry wt Kernels					
	Oil ($g\ kg^{-1}$)	Protein ($g\ kg^{-1}$)	Starch ($g\ kg^{-1}$)	50 mls (g)	50 mls^{-1}	Kernel dry wt (mg)	Germ (%)	Endosperm (%)	Germ Oil ($g\ kg^{-1}$)
B73 (normal)	48	120	648	30.1	141	222	14.5	85.5	321
Mo17 (normal)	41	131	676	30.6	100	307	12.2	87.8	280
AEC342	69	149	638	30.6	135	236	16.2	83.8	424
AEC335	99	138	639	31.9	165	206	21.8	78.2	492
AEC552-3	84	139	650	32.3	153	242	22.8	77.2	432
UHO51 136	137	594	31.3	142	227	23.3	76.7	38.8	551
UHO214	153	152	579	28.0	154	192	25.1	74.9	477
UHO416	122	155	570	28.4	141	205	23.9	76.1	530
UHO523	146	147	577	30.7	157	217	23.7	76.3	517
UHO44 127	145	587	30.1	210	168	22.1	77.9	35.6	528
Mean normals	44	126	662	30.4	121	265	13.4	86.7	301
Mean high oil	117	145	539	30.4	157	212	22.4	77.6	431
LSD 0.05	18	13	18	1.4	14	30	6.5	6.5	32
CV	10.4	5.5	1.7	2.7	5.5	6.0	18.8	4.8	12.0

The data indicate selection only for increased oil concentration can result in a correlated responses for germ and endosperm size, changing the ratio of germ to endosperm, and increasing the rate of oil deposition in the scutellum during kernel development. Selection for an increase in only total oil concentration in corn could indirectly select for one of the above traits. The trait responding to selection would be the trait with the highest heritability, which would probably be kernel size or germ size, so a decrease in kernel size is expected with an increase in total oil concentration unless a selection index is used that would put more weight on other traits.

B. Historical Review of High-Oil Breeding Programs

Several high-oil corn breeding programs at public institutions and commercial hybrid corn companies have been started since the late 1940s.[5,24–32] Most of the programs produced corn hybrids with oil values in the 6 to 7% range. One of the early high-oil corn breeding programs was started by C. M. Woodworth and continued by R. W. Jugenheimer at the University of Illinois.[25,26] The program emphasized the backcross method of breeding and involved inbred parents used in the popular double cross hybrid (e.g., U.S. 13) of that time as recurrent parents. Usually a cross of IHOx inbred followed by one backcross to the recurrent parent was used followed by selfing and selection to homozygosity. Some crosses were backcrossed to the IHO parent and then selfed to produce lines of higher oil concentration. Visual selection was for increased germ size with a minimum number of analyses for oil concentration (using gravimetric procedures). A comparison of two of the better high-oil double cross hybrids for agronomic performance over a 5-year period produced by the program is presented in Table 5.2.

The average grain yield of the two high-oil hybrids is reduced about 5% compared with the normal hybrid (U.S. 13). Total grain protein concentration is about 8% higher than U.S. 13 and about equal in grain moisture at harvest and standability. The high-oil lines used in these hybrids were released but not grown extensively by farmers because of a lack of an adequate market.

Funks Seed Co. of Bloomington, IL started a high-oil corn breeding program in 1946.[28] The objective of the breeding program was to develop high-oil inbreds for use in hybrid production. Part

TABLE 5.2
Performance of High-Oil Hybrids (25), Years 1954–58

	IL. 6021 (R75xR76)x(R84xK4)	IL. 6052 (R78x38–11)x(R84xK4)	U.S. 13 (WF9x38–11)x(Hy2xL317)
Oil (g kg^{-1})	64	62	48
Protein (g kg^{-1})	111	114	104
Grain yield (t ha^{-1})	6.9	7.0	73
Grain moisture (g kg^{-1})	20	22	20
Standability (%)	83	81	86

Source: From Ref. 25. With permission.

of the breeding effort was to select for higher oil concentration in large isolation blocks of improved inbreds. Individual ears taken from plants with the most desirable agronomic traits were analyzed for oil content. The top 33 or 50% of the highest oil sibbed ears were bulked to produce the line. A small gain in oil concentration (from 58 to 63 g kg^{-1}) using this within line selection procedure was observed. The hybrids using these selected higher oil inbreds also showed only slight increases in oil values (range 50–63 g kg^{-1}). Some lines may have had residual heterozygosity, so minor selection gains for oil concentration could be made. Selecting for increased oil levels within relatively homozygous corn lines would not be a breeding procedure expected to produce major gains in oil levels. In addition, IHO was used as a source of high-oil genes to develop other high-oil inbreds in the 7 to 10% oil range. In the mid 1950's Funks produced three high-oil double cross hybrids (G100HO, G101HO, G102HO) for sale. These high-oil hybrids were produced by crossing a high-oil single cross with a normal high performing single cross. The high-oil double cross hybrids were about 10% lower in grain yield than G75A, a widely grown double cross of the 1950s.[28] Jump states "our experiences have been that whenever IHO has been included in the background of a new inbred we have great difficulty in developing inbreds that have top performance in hybrid combinations."

Watson and Freeman[29] relate an additional high-oil corn breeding effort at Funks Bros. Seed Co. after it was purchased by C.P.C. Inter. Inc. in 1968. The uniqueness in this high-oil corn breeding program was the application of wideline nuclear magnetic resonance spectroscopy (NMR) to select single corn kernels for oil concentration. The procedures for using NMR analysis to determine the oil concentration in corn grain had previously been established.[33, 34] The pedigree and backcross methods of breeding were used with intense selection for agronomic traits during inbreeding. Tandem selection was used in a segregating population of about 1280 plants, selecting the best 150 for agronomic traits, and from these selecting the best 75 ears for oil levels. Kernels from these selected ears (100 or 200 kernels) were analyzed using NMR and highest 32 kernels in oil concentration selected. High-oil inbreds were developed and during 1971 and 1972 several high-oil hybrids in the 60 to 70 g kg^{-1} oil range were produced on contract on a substantial acreage near Pekin, IL. The hybrids performed well in 1971 but drought stress during August of 1972 reduced grain yields of the high oil hybrids much more than normal.[29] The high-oil corn breeding program was discontinued in 1972 when C.P.C. Inter. Inc. sold the company.

A high-oil corn breeding program using recurrent selection procedures to increase oil concentration in synthetics and then ear to row selection with selfing to develop high-oil inbreds was started by D. E. Alexander at the University of Illinois in 1956.[24,30,31,35] The initial objective was to create several germplasm sources different than IHO. Alexho synthetic was developed by random mating 56 open-pollinated corn cultivars in 1956. Phenotypic recurrent selection was used for several cycles to select for increased oil concentration and improved disease resistance. Development of NMR analyses in 1964 for bulk and single kernel samples made it possible to assay a large number of samples for oil concentration.[35] From September 1965 to May 1966 50,000 analyses for oil concentration were made by NMR (D. E. Alexander, pers. commun.). NMR analysis made

TABLE 5.3
Description of High-Oil Maize Synthetics Developed by D. E. Alexander, Department of Agronomy, University of Illinois at Urbana-Champaign, Urbana, IL.[36]

Synthetic	Description
R Alexho (M) C_{16}	Random mated 56 open-pollinated cultivars for two generations. Population was selected five cycles for oil content and stalk-rot. Single kernel NMR mass selection for oil content for 11 cycles. Mean oil content cycle–16 13.5%.
R D.O. (S_1) C_6	Recurrent selection for oil content and stalk rot. The synthetic was developed from 31 inbreds and individual ears of four Wrenham synthetics. Mean oil content cycle-6 9.5%.
R Alexho Elite (M) C_2	Seven high-oil lines from R Alexho (R804, R805, R806, 24, 612, 144, SK381) were selected for combining ability with an inbred tester. Selection was for increased oil content and resistance to Northern leaf blight, *Exserohilum turcicum* (Pass.) Leonard and Suggs. Mean oil content cycle-2 8.9%.
R Iowa 2-ear (M) C_2	Single kernel (10,000 K) from BS10(FR)C_2 were analyzed by NMR for oil content. 125 kernels were random mated for cycle–1. Single kernel selection for oil content within 125 families was used to produce cycle-2. Mean oil content of cycle-2 5.9%.
RHORYD(M)C_5	Selection for oil content in Armel's strain of Reid Yellow Dent. NMR single kernel analysis was used to select for oil content (400 ears with 300 kernels ear^{-1} assayed in cycle-0, 1, 2, 3, in cycle-4 100 kernels ear^{-1} used). Mean oil content cycle-5 8.6%.

Source: From Ref. 36. With permission.

possible the development of several improved synthetics with increased oil levels and improved agronomic traits. The description of the developed synthetics are listed in Table 5.3.[36]

The performance of high-oil hybrids using inbreds developed from recurrently selected Alexho Elite synthetic for increased oil concentration and grain yield by D. E. Alexander is presented in Table 5.4.[24] The average grain yield for the high-oil hybrids (11.1 vs. 11.9 t ha^{-1}) is about 7% lower than the normal check hybrid (Mo17 × B73). The oil concentration of the high-oil hybrids averaged about 1.7 times greater than the check hybrid (77 vs. 45 g kg^{-1}). Total protein concentration is slightly higher for the high-oil hybrids (104 vs. 101 g kg^{-1}). Grain moisture at harvest of the high-oil hybrids is higher than the check hybrid by about 4.2% absolute. The data indicate high-oil inbreds can be developed (in the 60 to 80 g kg^{-1}) from improved synthetics and used to produce high-oil hybrids with added value that are competitive with good commercial hybrids on a value added basis.

TABLE 5.4
Performance of High Oil Hybrids Urbana, IL 1984–87[24]

Pedigree	Yield (t ha^{-1})	Grain moisture (g kg^{-1})	Oil (g kg^{-1})	Protein (g kg^{-1})
R806 × B73	10.7	23	71	100
$AEC_2$7 × B73[a]	11.6	23	81	110
$AEC_2$342 × B73	11.2	25	74	107
$AEC_3$536–7 × B73	11.0	26	84	101
$AEC_2$183 × B73	11.4	24	76	101
Mo17 × B73	11.9	20	45	101

[a] AEC_2 = Alexho Elite Synthetic cycle-2.

Source: From Ref. 24. With permission.

In addition to the added value of high-oil content, high-oil corn hybrids also have the following added value traits: (1) high-oil corn has nutritional advantages for feeding because of greater energy per unit of feed; (2) protein concentration in high-oil hybrids has slightly enhanced protein quality because of the larger scutellum size compared with normal corn, and an increase in the amino acid lysine (dry wt.) can be from 0.28% for normal corn to 0.32% for high-oil corn;[9] (3) high-oil hybrids usually have a higher proportion of yellow pigments (carotenoids, xanthophylls) which are beneficial in poultry rations; and (4) the high-oil trait can be identity preserved by using NMR, near infrared reflectance, or near infrared transmission procedures to monitor oil concentration of corn lots during transport from producer to user.

C. High-Oil Inbreds Developed from the Illinois Breeding Program

The high-oil corn breeding program started in the late 1940s by C. M. Woodworth and continued by R. W. Jugenheimer and D. E. Alexander has developed several high-oil inbreds listed in Table 5.5.[36,37] Of the 26 inbreds listed in Table 5.5, only 12 (46%) have been released, and these inbreds were not widely used because of a lack of wide-area testing and a market for the product.

Pfister Hybrid Corn Co., El Paso, Illinois started a high-oil breeding program in the early 1970s. Pfister Hybrid Corn Co. marketed high-oil single cross hybrids starting in the 1980s. In 1989 Pfister Hybrid Corn Co., DuPont, and the University of Illinois entered into a joint project to develop and

TABLE 5.5
High-Oil Corn Inbreds Developed by the Illinois Agricultural Experiment Station From 1930 to 1974

Inbred	Pedigree	Breeder
R43	IHO	R. O. Snelling
R44	IHO	R. O. Snelling
R75*	(IHO × WF9)WF9	R. W. Jugenheimer and E. M. Woodworth
R76*	(IHO × 38–11)38–11	R. W. Jugenheimer and E. M. Woodworth
R78*	(IHO × Hy)IHO	R. W. Jugenheimer and E. M. Woodworth
R84*	(IHO × 187–2)IHO	R. W. Jugenheimer and E. M. Woodworth
R80	(IHO × L317)IHO(2)	R. W. Jugenheimer and E. M. Woodworth
R82	(IHO × 187–2)IHO(2)	R. W. Jugenheimer and E. M. Woodworth
R88	(IHO × L317)L317(2)	R. W. Jugenheimer and E. M. Woodworth
R89	(IHO × L317)L317(2)	R. W. Jugenheimer and E. M. Woodworth
R92	(IHO × WF9)WF9(2)	R. W. Jugenheimer and E. M. Woodworth
R93	(IHO × 187–2)187–2(2)	R. W. Jugenheimer and E. M. Woodworth
R94	(IHO × 187–2)187–2(2)	R. W. Jugenheimer and E. M. Woodworth
R118	(IHO × L317)L317(2)	R. W. Jugenheimer and E. M. Woodworth
R121	(IHO × 38–11)IHO(2)	R. W. Jugenheimer and E. M. Woodworth
R122	(IHO × L317)L317(2)	R. W. Jugenheimer and E. M. Woodworth
R123	(IHO × 187–2)IHO(2)	R. W. Jugenheimer and E. M. Woodworth
R124	(IHO × WF9)WF9(2)	R. W. Jugenheimer and E. M. Woodworth
R182*	(R75 × Oh51a)	R. W. Jugenheimer
R197*	(R80 × K201)	R. W. Jugenheimer
R801*	(B14 × IHO)B14(2)	D. E. Alexander
R802*	(B37 × IHO)B37(2)	D. E. Alexander
R803*	(C103 × IHO)C103(2)	D. E. Alexander
R804*	Alexho Synthetic	D. E. Alexander
R805*	Alexho Synthetic	D. E. Alexander
R806*	ASK Synthetic	D. E. Alexander

Notes: IHO = Illinois high-oil strain; * = released inbreds; (2) = two backcrosses to the recurrent parent.

market high-oil corn hybrids. Development of the Topcross®* method for producing high-oil corn in the early 1990s was a major factor in market expansion of high-oil corn. The Topcross method consists of mixing seed from a cytoplasmic male sterile single cross hybrid with normal oil levels with seed of a high-oil male fertile pollinator (usually a synthetic variety). When this seed mixture is planted, about 92 to 94% of the plants are male sterile and 6 to 8% are the male fertile high-oil pollinator plants (pollinators usually range from 120 to 150 g kg^{-1} in oil).

There were about 70 hybrid seed corn companies in 1997 producing and marketing Topcross seed. There were about 607,287 h (1.2 million acres) of high oil corn grown in the U.S. in 1998. (pers. commun.). Grain yields of Topcross hybrids grown under high productivity levels can equal the yields of normal hybrids[38] and can yield 5% less on the average in less productive environments.[38] A problem with high oil Topcross production is the tendency for growers to plant at normal plant densities. To compensate for the lower grain yield of the high-oil pollinator, higher planting densities are needed in Topcross production to maximize yields. Growers usually have fewer Topcross hybrids to plant, so selection of the "best" hybrid for a specific environment is more difficult. In addition, increased management for diseases and insects is necessary to protect the low frequency of pollinator plants. However, over time corn breeders will be able to improve on the performance of the pollinator.

The success of the Topcross method is based on the premise that xenia effects for oil "trick" the plant into producing more oil in the kernel but does not lower grain yields as much as high-oil single cross hybrids. To simulate the Topcross method nine different single cross hybrids were pollinated by a normal oil and high oil pollinator (Table 5.6). The four normal oil hybrids, averaged over 3 years, produced oil concentration in the 60 to 70 g kg^{-1} range (dry wt.) when pollinated by the high oil pollinator with no significant yield loss. The same four normal oil hybrids produced oil concentrations in the 47 to 55 g kg^{-1} range with similar yields when pollinated by the normal oil pollinator.[38] Four high-oil single cross hybrids produced yields about 15% lower than normal hybrids when pollinated by the high-oil pollinator and 12% lower when pollinated by the normal oil pollinator. The increases in oil concentration of normal oil hybrids pollinated by the high-oil pollinator results from an increase in germ weight, an increase of oil concentration in the germ, a reduction in percent endosperm (Table 5.6), and small changes in germ size (data not shown). These changes in kernel traits do not seem to reduce grain yields of the normal hybrids but do increase oil levels. The data support the premise that the Topcross method of high-oil corn production results from "tricking" the plant into producing greater amounts of oil in the germ with only small effects on grain yields.

D. Environmental Effect on Oil Concentration

Environmental factors can affect oil levels of high-oil corn hybrids, although the genotype effect on oil values is larger. Welch[39] found N, P, or K applications alone had very small effects on oil levels, but increased grain yields increased oil produced per hectare. Genter et al.[40] found N, P, and K increased grain yields but had no effect on oil content. A study in Georgia[41] for 2 years, five locations in 1962 and six locations in 1963 along with several planting dates at one location, found genotype had a greater influence on oil values than planting dates, locations, and years.[41] A later study in Georgia[42] using different N levels and boron had no effect on oil levels on the hybrids tested. These studies used corn hybrids in the 4 to 5% oil range.[39,40,41] Testing is needed on high-oil hybrids in the 7 to 10% oil range over locations and years to determine the affect of environmental factors on higher oil levels. An additional need is to conduct studies on the affect of supplemental fertilizers (N, K, P) on the oil levels of high-oil hybrids. Supplemental nitrogen application to high-oil hybrids could change the grain protein levels resulting in oil and starch changes. Evaluation of performance stability of high-oil hybrids is needed because of the lack previous of wide-area testing

* Topcross is a registered trademark of Optimum Quality Grains, Des Moines, IA.

TABLE 5.6
Means of 20-kernel Samples for Five Kernel Traits for Seven Maize Hybrids Pollinated by a High-Oil Pollinator (HOP) and a Normal Oil Pollinator (NOP) in 1996[a]

	Oil concentration (g kg^{-1})				Proportion of kernel, By Wt. (%)				Dry Wt. (mg)			
	Germ		Endosperm[c]		Germ		Endosperm[c]		Germ		Endosperm[c]	
Hybrid	**HOP[b]**	**NOP**	**HOP**	**NOP**	**HOP**	**NOP**	**HOP**	**NOP**	**HOP**	**NOP**	**HOP**	**NOP**
Normal Oil												
LH192 Sdms × LH82	430	376	16	13	15.9	13.4	84.2	86.6	40.3	33.0	264	263
LH192 Sdms × LH126	408	356	15	12	14.7	12.2	85.3	87.8	44.9	36.2	260	261
LH119 Sdms × LH211	402	343	13	10	14.8	12.1	85.2	87.9	44.9	37.3	259	272
LH132 Sdms × LH213	395	333	15	12	15.2	12.1	84.8	87.9	52.8	40.4	294	293
Mean	409	352	15	12	15.2	12.5	84.9	87.6	49.6	36.2	268	262
High Oil												
LH198 × $AEC_2$342	421	375	17	10	16.4	14.2	83.6	85.8	51.5	44.9	264	272
LH198 × $UHOC_3$350	486	446	17	17	18.9	16.8	81.7	83.2	54.1	48.5	232	240
Mean	454	411	17	14	17.7	15.5	82.7	84.5	52.8	46.7	246	256
Check												
LH192 × LH123	410	358	14	13	14.7	11.8	85.3	88.2	44.0	35.3	255	264
Mean	422[d]	370	15_{NS}	12	15.8[d]	13.2	84.3[d]	86.8	47.5[d]	39.4	261[d]	266
LSD (0.05)	21		—		2.1		2.3		6.3		8	
CV%	6.5		1.0		4.6		7.1		5.1		3.1	

[a] The HOP was Alexho single-kernel synthetic Cycle 20 ($ASKC_{20}$); the NOP was LH192 × LH123.
[b] HOP = High oil pollinator; NOP = normal oil pollinator.
[c] Endosperm + tip cap + pericarp.
[d] Significant at the 0.05 and 0.01 probability levels, respectively.

Source: From Ref. 38. With permission.

during their development. All these important factors need to be evaluated before large scale production of high-oil single cross hybrids is successful.

E. Xenia Effects

Xenia describes any immediate effect a pollen grain has on the germ or endosperm of seed plants. There are several reports on xenia effects in corn for several traits. Several reports have demonstrated xenia effects exist in corn for total oil levels.[43–47] An early detailed study on xenia effects in corn by Miller and Brimhall using lines developed from IHO and ILO (white endosperm) and three yellow endosperm lines (Corn Borer Syn. No. 1, Krug, and I198), along with mixed pollen pollinations of these lines, measured xenia effects for oil content in corn.[44] When ILO was used as female parent mean oil content of the ILO × ILO was 0.88%. For the ILO × yellow endosperm cross mean oil content was 1.81% or an increase of 0.93%. When the female parent, with an average oil level of 4.4%, was pollinated by a high-oil parent, the mean oil level was of 6.3%, or 1.9 unit increase. Their conclusion from the study was that the female parent had the largest influence on kernel oil concentration and the pollen source (xenia) had a smaller effect on oil levels. The xenia effects resulted from an increase in germ size and increased oil concentration in the germ (Table 5.7).[44]

Data in Table 5.7 illustrate that, as oil level of the pollen source increases, the proportion of germ in the kernel increases. This same relationship exists for oil concentration in the germ which increases with the increase in oil concentration of the pollen grain. Additional xenia effects were obtained in two additional studies.[45, 47] Garwood et al.,[47] using IHO and ILO strains and seven normal inbreds, found maternal effects on total oil concentration, oil in the germ, and the proportion of germ in the total kernel. These effects were attributed to the physiology of maternal parent. Xenia effects similar to those previously reported were also observed. Small cytoplasmic effects were observed for total oil concentration, but these were much less important than maternal and xenia effects. The xenia effects in high oil corns can cause problems growing high-oil performance trials with hybrids ranging in oil levels from 4 to 10%. Unless hand pollinations (selfs or sibs) are made for each hybrid, accurate estimates of the potential oil levels cannot be made. Under open

TABLE 5.7
The Immediate Effect of Source of Pollen Upon Proportion of Germ and Percentage Oil in the Germ of the Corn Kernel

Female Parent	Pollen Source	Proportion of Germ	Oil in Germ
ILO[a]	ILO	5.4	14.0
ILO	I198	7.4	21.0
ILO	IHO	10.0	30.0
I198	ILO	10.0	31.0
I198	I198	12.0	31.0
I198	IHO	15.8	39.0
IHO[b]	ILO	14.6	41.0
IHO	I198	19.8	45.0
IHO	IHO	23.1	50.0

[a] ILO = Illinois low-oil strain.
[b] IHO = Illinois high-oil strain.

Source: From Ref. 44. With permission.

pollinated field conditions, oil levels of the lower oil hybrids will be increased and oil levels of the higher oil hybrids will be reduced because of xenia effects. To accurately measure the oil levels of a hybrid in a performance trial, plants in several replications must be hand-pollinated or "sideline" plots used to prevent xenia effects so that accurate values of the oil concentration can be obtained for a hybrid.

V. SELECTION PROGRESS FOR INCREASED OIL CONCENTRATION IN CORN

There are numerous reports on the response of IHO to selection for increased oil concentration in corn.[3,48–55] Additional selection programs to increase the oil levels in germplasm pools other than IHO also have been reported.[56–61] The first, and longest continuing, plant selection program to change the chemical composition of the corn kernel was started by C. G. Hopkins of the University of Illinois in 1896.[48] The open pollinated cultivar 'Burr's White' (white endosperm) was used to initiate selection for high and low oil and protein concentration in the corn kernel. There are a number of reports summarizing selection progress during the course of this experiment and no attempt is made to summarize all the published data for increased oil concentration from this experiment. A summary of the selection response for increased oil concentration through 90 cycles of mass selection in IHO was reported by Dudley and Lambert.[55] The mean oil level of the IHO strain has increased from 4.7% in 1896 (cycle-0) to 19.3% in 1989 (cycle-90). Selection procedures varied during the 90 cycles, so the experiment can be separated in four segments.[53] During 90 cycles of selection for increased oil concentration the average linear rate of change was +0.19% per oil cycle with linear regression values ranging from +0.22% per oil cycle (cycle 0 to 9) to +0.14% per oil cycle (cycles 26 to 52). Realized heritability values ranged from 0.34 to 0.11 for different segments.[55]

The large increase in oil concentration over 90 selection cycles is even more amazing when the origin of the IHO strain was determined. Winter showed the IHO pedigree of cycle-28 traced back to a single maternal ear.[49] This could mean a large amount of genetic variability can be contained in a small population of 60 individuals to account for the selection progress. The gains for IHO after 90 cycles of selection in terms of the additive genetic standard deviation was $22\sigma_A$.[55] Dudley and Lambert showed significant amounts of genetic variation was present in the IHO strain in cycle-65.[62] Dudley has inferred, based on estimates of the additive genetic variance, that all the selection progress can be accounted for by the additive genetic variance in IHO.[54]

A. Estimates of Gene Number Controlling Oil Concentration

Estimates of the number of loci controlling oil concentration in IHO and ILO have been made.[54,55,62–65] Estimates of gene number in the oil strains range from 33 loci in cycle 28,[64] 20 to 40 loci by Sprague and Brimhall in 1949,[65] 54 loci in cycle 76,[54] to 69 loci in cycle 90.[55] Estimates of the number loci controlling oil concentration have increased as cycles of selection and progress in IHO and ILO strains have increased, because the estimates are a function of selection progress and additive genetic variance. The experiment does reveal a large increase in oil concentration is possible in corn with a rather small number of genes and a small population size.

B. Phenotypic Recurrent Selection for Increased Oil Content

Sprague et al.[56,57] selected for increased oil concentration in three different crosses. Phenotypic recurrent selection was used to select for increased oil levels in the crosses IHO × wxOs 420, I198 × Hy, and Stiff Stalk Synthetic with one, two, and three cycles of selection, respectively. The IHO × wxOs 420 population increased from a mean 72 g kg^{-1} to 105 g kg^{-1} oil in two cycles, I198 × Hy increased from a mean of 42 g kg^{-1} to 51 g kg^{-1} in one cycle,[56] and Stiff Stalk Synthetic from a mean of 42 g kg^{-1} to 70 g kg^{-1} in three cycles of selection for increased oil concentration.[57]

Comparing the progress of five generations of selfing within these three populations to progress from phenotypic recurrent selection showed the selfing series increased oil concentration 0.13% per cycle vs. 0.41% per cycle for recurrent selection.[57]

C. Single Kernel Selection for Increased Oil Content

In 1956, Alexander[24] developed Alexho Synthetic, by random mating 56 open pollinated corn cultivars, and initiated a recurrent selection program for increased oil levels. The main reason for developing Alexho Synthetic was a lack of genetic relationship to IHO. From cycle 0 to cycle 4 phenotypic recurrent selection for increased oil levels was used by selfing about 1000 plants each cycle, inoculating with stalk-rot, and saving 200 to 300 ears to assay for oil content. The ears with the highest oil levels (S.I. = 20%) were recombined to form the next cycle. From cycle-5 through cycle-28 single-kernel selection was used to increase oil content.[60] A half-sib recurrent mass selection method was used with 125 half-sib families used each cycle. The three kernels from each ear with the highest oil content were planted ear-to-row. From cycle-5 through cycle-9, 200 kernels per ear were assayed for oil content; cycle-10 through cycle-13, 100 kernels per ear; and cycle-14 through cycle-28, 50 kernels per ear. Evaluation of selection progress through 24 cycles was for 2 years in the U.S. and Yugoslavia.[61] Oil concentrations increased from a mean of 51 in cycle-0 to 170 g kg^{-1} in cycle-24. Crosses of certain cycles to the inbred testers B73 and R802A showed increases in oil concentrations of 53 g kg^{-1} to 107 g kg^{-1} for B73 and 63 g kg^{-1} to 124 g kg^{-1} for R802A. Testcross data indicated inbreds developed from later cycles of selection could be used in crosses to inbreds with normal oil levels for the production of high oil hybrids. Grain yield was reduced over 24 cycles of selection from 4.8 t ha^{-1} for cycle-0 to 3.2 t ha^{-1} for cycle-24 or about 62.7 kg ha^{-1} $cycle^{-1}$ for the population *per se*. Grain yields of testcrosses to the testers showed less yield reduction per cycle. Grain moisture at harvest, kernel-row number, and oleic fatty acid content increased with selection for increased oil concentration. Stalk lodging, ear length, 500-kernel weight and linoleic fatty acid decreased over 24 cycles of selection in the population *per se*.

An additional evaluation of Alexho Synthetic cycles 0, 5, 10, 15, and 20, selected only for increased oil concentration, had an increase from 51 g kg^{-1} in cycle 0 to 135 g kg^{-1} in cycle 20 with a significant rate of increase of +4.1 g kg^{-1} $cycle^{-1}$.[63] Associated with the oil increase was a significant decrease in starch concentration from 664 g kg^{-1} in cycle 0 to 593 g kg^{-1} in cycle 20 or a decrease of –3.5 g kg^{-1} $cycle^{-1}$. The number of kernels in 50 mls showed a significant increase from 139 kernels in cycle 0 to 162 in cycle 20, or +1.2 kernel increase per cycle. The decrease in kernel size was associated with a significant change in the endosperm to germ ratio. The ratio decrease from 86% endosperm/14% germ in cycle 0 to 75% endosperm/25% germ in cycle 20. Lambert et al. concluded that to produce agronomically acceptable maize breeding lines selection for increased oil concentration should also include selection for starch and protein concentrations plus kernel size using some form of tandem or index selection.[63]

D. High Intensity Selection for Increased Oil Concentration

Miller et al.[59] used high intensity selection to increase the oil concentration in the open pollinated cultivar 'Reid Yellow Dent'. Seven cycles of half-sib intra-ear (single kernel) mass selection were completed using 400 ears per cycle and assaying 300 kernels per ear for cycle-0 through cycle-3 and 100 kernels per ear for cycle-4 through cycle-7 using NMR procedures for oil determination. The five kernels with the highest oil concentration were selected from each ear and planted ear-to-row. Pollen from the plants with the highest oil content in each half-sib family was bulked and used to pollinate the remaining plants in all families. The percentage of selected individuals from cycle-0 through cycle-3 was 0.33% and from cycle-4 through cycle-7 was 1.0%. Oil concentration increased from a mean of 40 g kg^{-1} in cycle-0 to 91 g kg^{-1} for cycle-7 or about 0.66 g kg^{-1} $cycle^{-1}$. Estimates of genetic variances for cycle-0 and cycle-5 showed a nonsignificant change in the

additive genetic variance (0.014 vs. 0.016). No significant dominant genetic variance was estimated for either cycle-0 or cycle-5. Nonsignificant differences were observed for changes in grain yields over the seven cycles when evaluated over 3 years. Predicted gains from within half-sib family mass selection, and the combined selection among and within half-sib families, showed the largest gains from selection can be expected when a higher selection intensity is used within half-sib family selection.[59]

E. Modified Phenotypic Recurrent Selection for Increased Oil Concentration

Trifunoviÿ and Dumanoviÿ[58] used a "modified" phenotypic recurrent selection method to increase the oil concentration in three Yugoslavian corn synthetics, U-1, U-2, and U-3. The three synthetics were developed from inbreds, lines, and local varieties with above-average oil concentration in the kernels. The mean oil concentrations for cycle-0 was U1 = 53 g kg^{-1}, U2 = 55 g kg^{-1}, and U3 = 56 g kg^{-1}. The modification of phenotypic recurrent selection method was in the pollinator of the selected individuals for intermating. Instead of using pollen only from selected individuals for intermating each cycle, pollen from additional genotypes unrelated to the synthetic were added to the selected group. Their selection criteria was that oil content had to be 1 to 5% higher than selected plants in the synthetic(s). They designated the procedure the "pollinator method." A comparison of three cycles of classical phenotypic recurrent selection with the "pollinator method" showed cycle 0 = 56 g kg^{-1} and cycle-3 = 65 g kg^{-1} vs. cycle-0 = 56 g kg^{-1} and cycle-3 = 81 g kg^{-1} for the two methods, respectively. The classical procedure had a 0.38% increase in oil concentration per cycle and the pollinator method 1.77% per cycle increase, or about a four-fold increase for the pollinator method in gains per cycle. Evaluation of oil increases in the other two synthetics showed U2 cycle-0 = 58 g kg^{-1}, cycle-3 = 80 g kg^{-1}; U3 cycle-0 = 55 g kg^{-1}, cycle-3 = 108 g kg^{-1}. There are some problems with the interpretation of results, because population size and selection intensity were not constant over populations and cycles, but the observed gains were rather spectacular. The large increase observed for the "pollinator method" may have resulted from selection for xenia effects.

The five selection experiments for increased oil concentration in corn showed oil to be highly heritable, that oil concentration will respond to several selection methods, and that changes per cycle of 0.5% to 0.8% in oil can be expected for most populations where appropriate analytical procedures (NMR, NIR, or NIT) are used. Additional data are needed on selection for increased oil concentration along with selection for grain yield and other agronomic traits to determine if the observation of Miller et al.[59] relative to no grain yield decrease with an increase in oil concentration. Selection in corn synthetics for only increased oil concentration will usually result in an increase in germ size, germ oil concentration, smaller kernel size, and reduced grain yields. Selection indices involving genetic correlations among these traits are needed so inbreds developed from the improved synthetics can produce high-oil hybrids with good economic yields. Effective population size, selection intensity, inbreeding effects, genetic drift, and linkage can affect grain yield decreases with increased oil levels.

F. Association of Oil Concentration with Agronomic Traits

Correlation estimates of increased oil levels with grain yields and other agronomic traits in high-oil corn have been made.[30,44,53,65–70] In general, grain yields of high-oil hybrids usually decrease at oil levels greater than 8%. Evaluation of 49 high-oil hybrids (range in oil values 3.8 to 10.2%) found a correlation between grain yield and oil values of $r = -0.35$ (significant at P ð 0.01). The correlation of 29 of these hybrids (range 5.4 to 8.4%) between grain yields and oil values was $r = 0.06$.[30] Miller and Brimhall[44] found variation in total oil concentration of corn was not associated with total protein content, but was positively correlated with percent germ protein and relative

concentration of tryptophan in the kernel. The latter association may be one reason for the enhancement of high-oil corn in animal diets. Association of increased oil concentration with reduced ear length, smaller ear diameter, lighter kernel weight,[3,53] reduced plant and ear height,[53,59] and earlier flowering[50] have been reported. In two corn synthetics (D.O. Syn. and Alexho) selected for one and two cycles for increased oil concentration, researchers found no association among 866 ears between oil concentration and kernel weight.[69]

The association of a reduction in grain yields with increased oil levels may relate to the mutual exclusiveness of oil concentration and grain yield. Supporting this concept is the knowledge that the synthesis of a fixed amount of oil requires twice the energy as the same amount of starch.[46] The genotype of the sporophyte greatly determines the amount of oil produced by high-oil hybrids so these hybrids may have a more efficient energy trapping system. Alexander and Lambert[46] designed an experiment, using xenia effects to alter the oil concentration of the same genotype or hybrid by pollinating with male parents with different oil concentrations. They found one hybrid out of seven produced significantly more calories per plant with a high-oil male parent compared with a low-oil pollen parent. Five of the seven hybrids did not differ in calorie trap per plant, and one hybrid produced significantly less calories per plant. The inference was that oil content and calorie yield per plant are probably not associated in some hybrids, but calorie yields in other hybrids could be limited by an inefficient oil synthesizing system.

Dudley, et al.[70] evaluated hybrid crosses among the nine Illinois chemical strains for oil, protein content, and calorie production. A negative correlation between grain yield and oil content (r = –0.49 (significant at P ð 0.01)) (oil 29 g kg^{-1} to 162 g kg^{-1}; yield 3.1 to 7.9 t ha^{-1}) was observed. Calories per gram of dry matter was determined largely by oil concentration. Calories per kernel or per hectare was determined largely by kernel weight or grain yield. The negative associations in these materials, which have been selected only for oil and protein concentration, may be due to undesirable linkages among genes controlling oil concentration, kernel size, and kernel weight. A large amount of research on high-oil corn involves IHO, which could bias results because selection may have reduced genetic variation for other agronomic traits other than oil concentration. New corn synthetics need to be developed with good agronomic traits, but using lines with no previous history of selection for increased oil concentration to initiate multiple trait selection with emphasis on increased oil concentration.

G. Inheritance of Oil Concentration in Corn

Winter was the first to use certain statistical estimates to measure quantitative variation in oil concentration in the Illinois chemical strains.[49] Sprague and Brimhall[65] were the first to evaluate segregating generations (F_2, BC_1 P_1, BC_1 P_2) involving crosses of IHO, ILO, and normal inbreds to determine the inheritance of oil levels in corn. Segregating generations of IHO × ILO cross showed dominance for low oil concentration; other crosses showed partial dominance or dominance for high oil levels. Analysis of a diallel among the Illinois chemical strains showed dominance for both high and low oil concentrations.[70] Estimates of total genetic variance for oil concentration in 'Reid Yellow Dent' showed no dominant genetic variance but mostly additive genetic variance.[59] Moreno-Gonzalez et al.[66] measured linkage effects in IHO × ILO cross. The F_2 and F_6 generations, derived by random mating, were used in a Design III mating to estimate genetic variances. The additive genetic variance estimate was about seven times larger than the dominance variance in the F_2 ($V_A = 1.550$, $V_D = 0.220$) and about four times larger in the F_6 ($V_A = 0.812$, $V_D = 0.189$). Because additive genetic variance in the F_6 was about 50% of the F_2, the inference was that loci controlling high-oil concentration in the kernel were in coupling phase linkages in IHO and ILO. The lack of linkage effects on the dominant genetic variance estimates indicated loci with dominant genes were distributed throughout the corn genome and were not linked, so they segregated independently. They also proposed that some genes were dominant for low oil concentration and other genes were dominant for high oil concentration.

H. Molecular Marker Linkage With Oil QTL

Several types of molecular markers are being used to detect linkage between marker loci and quantitative traits (QTL) loci in an attempt to enhance selection progress. Analysis of 200 S_1 families from a single F_1 plant over 2 years for the cross IHO by ILO (Early Maturity Selection) by Berke and Rocheford[71] revealed the complexity of the problem. Restriction fragment length polymorphism (RFLP) analysis was used for these 200 S_1 families at 80 polymorphic loci spaced about 24 centimorgans throughout the genome. They found 31 RFLP loci located in 11 regions were significantly associated with oil concentration. Major QTL for oil concentration were located on chromosomes 2, 5, 6, and 9. Of the 31 loci, 22 showed only additive gene effects, eight showed both additive and dominant gene effects, and one only dominant effects. For the nine loci showing dominant gene effects, two showed dominance for high oil concentration (from high oil) and seven for low oil concentration (from low oil).

All 22 alleles for high oil concentration showing additive gene effects came from the high-oil strain. Nine loci located in seven regions had significant marker genotype by environment interactions for oil concentration. In addition, major QTL for kernel weight were located on chromosomes 4 and 5. To further explain the genotypic and phenotypic variation observed for oil concentration and kernel size from this cross, stepwise multiple regression estimates were used. Seven RFLP loci on chromosomes 2, 3, 5, 6, 8, and 9 accounted for 54% of the total phenotypic and 61% for total genotypic variation for oil concentration. The data demonstrate the quantitative inheritance for oil concentration in this genetic background. Both parents had been selected for many generations (90 and 70) for increased or decreased oil concentration. Using these materials it would be difficult to combine these chromosome regions into one line to enhance oil concentration because of the large number of markers. However, additional data using different high-oil genotypes may be more useful for marker assisted selection for increased oil concentration. Development of genome sequencing and "chip technology" to determine gene function may lead to additional ways to enhance oil concentration in corn, with associated enhancements in kernel size, starch concentration, and grain yields.

VI. FACTORS CONTROLLING OIL QUALITY IN HIGH-OIL CORN HYBRIDS

A. Introduction

The quality of corn oil is an important consideration in the development of high-oil corn hybrids. Unrefined corn oil contains triglycerides (79%), sterols (4.5%), mono- and diglycerides (3.9%), hydrocarbons–sterol esters (2.9%), free fatty acids (1%), polar lipids (8.7%), and minor amounts of waxes, tocopherols, and carotenoids. The polar lipids contain phospholipids (66%), and glycolipids (34%).[5] Corn oil is of high quality due to the high amounts of polyunsaturated fatty acids. The composition of the triglycerides relative to fatty acid content determines the quality of corn oil or ratio of saturated to unsaturated fatty acids. Normal corn has an average fatty acid composition of 11% palmitic (16:0), 2% stearic (18:0), 24.1% oleic (18:1), 61.9% linoleic (18:2), and 0.7% linolenic (18:3).[5] In addition, minor amounts (<1%) of lauric (12:0), myristic (14:0), palmitoleic (16:1), arachadic (20:0), behenic (22:0), erucic (22:1), and lignoceric (24:0) have been isolated.[72–76] Methods used to measure corn oil quality include the ratio of the fatty acid linoleic (unsaturated) to the other major fatty acids in corn oil or the saturated fatty acids, and iodine number. The ratio unsaturated to saturated fatty acids would be about 1.67 for normal corn, 2.31 for sunflower, 1.36 for soybean, and 0.12 for palm oil, which contains large amounts of highly saturated fatty acids.

Iodine number, a measure of the degree of unsaturation of corn oil, has a negative correlation with total oil concentration.[67–69,77–80] This negative association of total oil concentration and iodine number was measured by Jump[77] for cycle 47 of IHO and ILO strains. The ILO strain (mean of 0.98 g kg^{-1} oil) had an iodine number of 134 and IHO (mean of 141 g kg^{-1}) had an iodine number

of 114. The negative association between oil concentration and iodine number results from a decrease in linoleic and an increase in oleic fatty acids.[75] Quackenbush et al.[76] evaluated 125 widely used U.S. Corn Belt inbreds with a range in total oil concentration from 12 to 57 g kg^{-1} and a range in iodine number of 111 to 151. The inbred with the lowest iodine number (111) was CI38B and the highest (151) was H31, which also had the lowest oil content (12 g kg^{-1}). The inbred R802 (B37 × IHO) B37 is an exception to the above observations, because it has an oil concentration of 70 g kg^{-1} and a linoleic content of 60%. High oil corn hybrids (ranging in oil content from 56 to 70 g kg^{-1}) have been developed with linoleic fatty acid contents in the range of 52 to 60%.[78–80]

B. Factors Affecting Fatty Acid Composition in Corn Oil

The two major factors affecting fatty acid composition of corn oil are genotype and environment. Fatty acid composition of commercial corn hybrids have varied some over the years, but changes probably result from the use of different inbreds in corn hybrids rather than environmental effects. The average iodine number for corn received at a midwest wet milling plant in 1955 was 126 and in 1961 was 124. Beadle et al.[79] showed linoleic fatty acid changed from 58.7% in 1964 to 61.9% in 1968. Weber[80] found the genotype caused changes in linoleic fatty acid content of commercial corn hybrids grown in the 1970s. In 1970, B37 (60% linoleic) was estimated to be involved in 26% of the hybrid seed produced along with other widely used inbreds W64A (63% linoleic) and Oh43 (66% linoleic). In 1975, A632 (67% linoleic) and Mo17 (68% linoleic) were estimated to be used in 22% of the hybrids produce indicating changes in the inbreds of corn hybrids could influence the fatty acid composition of commercial corn hybrids.

C. Affect of Environment on Fatty Acid Composition of Corn Oil

Jellum and Marion[41] evaluated fatty acid content at five locations in 1962 and six locations in 1963. The fatty acid contents ranged from 33.0 to 38.5% for oleic acid and 43.7 to 50% for linoleic. Planting dates at one location each year had no effect on oil quality. Significant effects were found for fatty acid composition for years, locations, and hybrids. The largest affect was for genotypes or hybrids with the ranks of hybrids for years and locations being similar. The range and total oil concentration (41 to 52 g kg^{-1}) of the nine commerical hybrids was small so it is difficult to infer similar results would be obtained for high-oil hybrids in the range of 70 to 90 g kg^{-1} total oil.

The fatty acid composition of the oil of the corn kernel is affected by position on the ear.[81] The fatty acid content of the oil increased for palmitic and linoleic for kernels from the base to tip of the ear. Oleic fatty acid content of kernels decreased from base to tip. Sampling kernels in the central portion of ear is recommended for samples to be analyzed for fatty acid content.

D. Genotypic Variation in Fatty Acid Composition of Corn Oil

Genetic variation in fatty acid composition of corn oil has been determined by evaluating a series of corn inbreds or by inheritance studies of corn genotypes that differ in levels of a particular fatty acid(s). Jellum[82] evaluated S_1 kernels of 1850 foreign introductions, 260 U.S. introductions, and 140 Australian inbreds for fatty acid composition. Ranges of 6 to 22% for palimitic, 0.6 to 15% for stearic, 14 to 64% for oleic, and 19 to 71% for linoleic fatty acids were found. Also, higher than normal levels for certain minor fatty acids were found in some of these materials. In general, the lines assayed had higher levels of saturated fatty acids than commercial corn oil. Unfortunately the corn lines with unusual levels of certain fatty acids were never used in a high-oil corn breeding program.

There is a tendency for corn inbreds developed in northern U.S. to have higher linoleic levels than inbreds developed in southern U.S.[31] Evaluation of 169 northern U.S. inbreds had an average linoleic acid content of 58% and 63 southern U.S. inbreds had an average content of only 48%.

E. Inheritance of Fatty Acid Composition in Corn

The first inheritance study on fatty acid composition in corn was by Poneleit and Alexander,[83] using lines developed from IHO. Evaluating single kernels in the F_2, BC_1P_1, and BC_1P_2 generations from the cross IHO × R84 they proposed a single recessive gene (ln) for high linoleic fatty acid. The results were probably due to the close genetic relationship between the parents IHO and R84 (IHO × 187–2) IHO plus the large difference in linoleic acid levels (IHO = 48.8%, R84 = 61.3%). The genetic model proposed the ln allele controlled the desaturation of oleic fatty acid at the Δ 12–13 position to linoleic. The results were supported by de la Roche et al.[84] showing the goodness of fit of the F_3 families from IHO × R84 cross to the single recessive gene model. De la Roche evaluated additional crosses and segregating generations of C103 × IHO and C103 × R84 and found a single gene controlled linoleic acid differences in the C103 × R84 cross. In the C103 × IHO cross at least two loci controlled the linoleic levels. Analytical procedures made these genetic studies possible. The assay of single corn kernels used gas liquid chromatography procedures that today could probably be done more efficiently using reversed-phase high-performance liquid chromatography. Inheritance studies for palmitic, stearic, oleic, and linoleic in three crosses Ab26A × X-187, 9-908A × R921E, and GE82 × R196 suggested a more complex mode of inheritance.[85] Using generation mean analyses and single kernel assays, additive gene action was the major type for palmitic, oleic, and linoleic, with stearic having about equal dominant and additive gene action. Further analyses of data showed differences in the parental levels of palmitic and oleic in segregates from the cross Ab26A × X-187 were each controlled by a single gene. The allele for high palmitic level in Ab26A was partially dominant to the allele in X-187 and the gene for high oleic showed complete dominance in this cross. Single gene control of linoleic in this cross was not observed probably because of the small difference in the levels in the two parents. A two gene model was proposed for linoleic fatty acid with one locus showing partial dominance and the other locus having no dominance for low linoleic in GE82 (35.2%) vs. high linoleic in R196 (70.8%). Jellum and Widstrom,[86] using inbreds derived from plant introductions that ranged in palmitic from 1.5 to 11.5%, found in segregating generations a single gene with dominance for low stearic levels. Similar studies by Sun et al.[87] using inbreds Ayx 187 (9.4% palmitic, 2.3% stearic) and Ay 499 (16.9% palmitic, 1.4% stearic) found Ayx 187 had a single dominant gene(s) for high stearic. The data for palmitic in this cross showed Ay 499 had dominant gene(s) for high palmitic but the results were less conclusive.

Several studies have located genes controlling fatty acid composition in corn oil using waxy marked reciprocal translocations, B-A translocations, monosomics, and trisomic genetic stocks. Plewa and Weber[88] used monosomic analysis to locate genes controlling fatty acid levels in corn. The methodology is to compare the fatty acid content of single kernels that are either monosomic or diploid. If gene(s) are located in a specific monosomic and the gene shows dosage effects, a lower fatty acid level is expected in the monosomic kernels compared with diploid control. To locate gene(s) controlling the conversion of oleic to linoleic fatty acid in corn, they crossed a stock high in oleic and low in linoleic to several corn monosomics. The mean oleic fatty acid level of the monosomic-2 germs was 40.5 ± 2.2% with the mean of diploid germs being 34.3 ± 0.45%. The linoleic fatty acid content in this stock was 32.93 ± 3.4% for monosomic-2 germs and the mean of the diploid sibs 46.8 ± 0.5%. The fact monosomic-2 subpopulations had a significantly higher percentage of oleic and significantly lower level of linoleic relative to diploid control was used to infer gene(s) controlling the conversion of oleic to linoleic fatty acid is in chromosome 2. No significant differences in the means for oleic and linoleic were found in the other monosomic or diploid sibs comparisons further supporting evidence the gene(s) was located in chromosome 2. Using trisomics 3, 4, 5, and 6 along with three B-A translocations involving chromosome 5 placed additional gene(s) controlling the conversion of oleic to linoleic in chromosome 5L. Widstrom and Jellum,[89] using testcross and F_2 generations of the waxy marked reciprocal translocation series involving 15 of the 20 chromosome arms each crossed to the inbreds GE82 and X-187, found gene(s) in chromosome 5L that controlled the conversion of oleic to linoleic fatty acid. They also

found in the inbred X-187 a recessive gene in chromsome 4L that controlled a high linoleic fatty acid level. They inferred this could be the location of the ln allele. Genetic modifiers were also located on chromosome 1S that affected the conversion of oleic to linoleic fatty acid.

Analyses of a diallel set of nine corn inbreds by Poneleit and Bauman,[90] for total oil concentration, palmitic, stearic, oleic, and linoleic fatty acids grown over 2 years, showed small environmental effects on total oil or oil quality. Significant general combining ability (GCA) and specific combining ability (SCA) effects were observed for the four fatty acids. The nine inbreds covered a good range of total oil values (23 to 73 g kg^{-1}), and for oleic (18.1 to 38.0%) and linoleic (47 to 71%) fatty acids. Analysis of SCA effects showed the inbreds Hy_2 and 411 had lower levels of dominant gene action for total oil concentration than the other seven inbreds. Inbred H60 had the largest dominance effects for palmitic and inbreds 591, C103, 411, and H60 had large dominance affects for high oleic and low linoleic. The diallel analysis indicated selection progress for any of the four fatty acids could be expected based on the estimates obtained.

Selection for increased oil concentration in corn may result in reduced levels of the unsaturated fatty acid linoleic because of the negative relationship between total oil concentration and oil quality. During 24 cycles of single-kernel recurrent selection in Alexho synthetic for increased oil content, nonsignificant changes were observed in the mean fatty acid content for linoleic (55.3 vs. 54.4%), and oleic (30.2 vs. 32.1%) for cycles 0 vs. 24. A small significant negative rate of change per cycle was observed for linoleic (b = –0.139 (significant at P ð 0.05)) and small positive change for oleic (b = +0.139 (significant at P ð 0.05)) for 24 cycles.[61] No significant changes were observed for palmitic or stearic. Pamin et al.[91] found reciprocal full-sib and S_1 line per-se selection were effective methods for increasing the total oil concentration in three synthetics. The S_1 *per se* selection had a significant increase in the linoleic fatty acid content in three cycles of selection. A high negative genetic correlation estimate between oleic and linoleic fatty acids (r_g = –0.96) was observed. However, moderate correlation estimates between total oil concentration and oleic (r = +0.51) and linoleic (r = –0.48) were also observed. They proposed that careful monitoring of fatty composition during selection for increased total oil could be done for several selection cycles without lowering oil quality.

Molecular marker data for fatty acids in the cross IHO by ILO (early maturity selection) was reported by Alrefai et al.[92] Two hundred S_1 families and 80 polymorphic RFLP markers spaced throughout the genome were used. Fifteen RFLP loci were clustered in 12 chromosome regions for plamictic, 17 markers in ten regions were associated with stearic, 12 markers in eight regions for oleic and linoleic and 17 markers in eight regions associated with linolenic. Multiple regression models using means from the 200 S_1 families found four RFLP loci explained 24% of the phenotypic and 62% of the genetic variation for palmitic. Five loci explained 51% and 71% of the phenotypic and genetic variation for stearic; three loci explained 67% and 79% of the phenotypic and genetic variation for oleic; two loci explained 67% and 81% of the phenotypic and genetic variation for linoleic; seven loci explained 52% and 78% of the phenotypic and genetic variation for linolenic. A single RFLP loci on chromosome 6 was linked to a QTL that explained 63% of the phenotypic variation for the ratio of oleic to linoleic which was in the region of the linoleic acid-1 gene. The data indicate fatty acid concentration in the corn kernel can be modified for the five major fatty acids through selection. The data indicate these are major gene(s) for the conversion of each fatty acids with modifier loci depending on the genotypes chosen for study. However additional information is needed on the nutritional and health value of specific fatty acids before a selection program is initiated to alter the fatty acid composition found in normal corn.

VII. VITAMIN E OR TOPCOPHEROLS IN CORN

A. Introduction

Another important component of high-oil corn hybrids is the Vitamin E or tocopherol levels of the hybrids. Tocopherols or Vitamin E function in corn oil to protect the double chemical bonds from

oxidation. Since Vitamin E is soluble in corn oil and prevents autoxidation, an increase in Vitamin E content could increase the shelf-life of corn oil. Also, Vitamin E is an essential component in animal diets and is becoming more important in human diets. The four tocopherol isomers found in corn oil are designated as α-, β-, γ- and δ- tocopherols.

Grams et al.[93] evaluated four corn hybrids (2 normal, 1 high-oil, 1 opaque-2) for tocopherol content. They found 70 to 80% of tocopherols were located in the corn germ and 11 to 27% in the endosperm. Most of α-tocopherol (94 to 96%) and γ-tocopherol (93 to 96%) was in the germ. The high-oil hybrid Alexho 144 × R802A had higher levels of α- and γ- tocopherols ($\alpha = 25.4$, $\gamma = 70.0$ μg g^{-1} d.m.) than a normal hybrid ($\alpha = 14.7$, $\gamma = 38.6$ μg g^{-1} d.m.). Quackenbush et al.[76] found a range in tocopherols from 0.03 to 0.33% of the oil in 125 inbreds surveyed. Another study, using 20 inbreds with higher oil levels, showed α-topcopherols varied from 9.1 to 64.6 μg g^{-1} d.m. and γ-tocopherols ranged from 13.6 to 128.7 μg g^{-1} d.m.[94] The range in α- and γ-tocopherols is positively correlated with total oil concentration.[95] A positive correlation ($r = +\ 0.60$) between the amount of linoleic fatty acid and Vitamin E per gram of oil for 18 dent corn hybrids has been observed.[95]

B. Genetic Variation

Evaluation of 100 S_1 families of RSSSC for total oil concentration (range 22 to 57 g kg^{-1}) and α- and γ-tocopherols over 2 years showed a range of 0.0 to 138.2 μg g^{-1} d.m. of embryo for α-tocopherol and 0.0 to 409.3 μg g^{-1} d.m. of embryo for γ-tocopherols.[96] The mean values were 61.4 and 194.4 μg g^{-1} d.m. of embryo for α- and γ-tocopherols, respectively. Two S_1 families out of 100 had no detectable level of α-tocopherol and one S_1 family had no detectable level of γ-tocopherol. There was a negligible effect of years (1980–1981) on α- and γ- tocopherols (245.8 vs. 265.7). A significant year by genotype interaction was observed for both tocopherols. Broadsense heritability estimates were $h^2 = 0.62 \pm 0.16$ for α- and 0.68 ± 0.15 for γ-tocopherol.[96] Estimates of genetic variances indicate recurrent selection should be effective for changing the levels of α and γ-tocopherols in RSSSC with appropriate monitoring procedures.

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6 Sweet Corn

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I. INTRODUCTION

Sweet corn is one of the most popular vegetables in the United States and Canada. Consumption is increasing in eastern Asia, Europe, and South America. In the U.S. it is a symbol of summer; consumers know the names of their favorite varieties, and each summer there are popular articles on the growing and preparation of sweet corn. When many Americans think of corn they think of sweet corn, seemingly not aware of the millions of acres of field corn that form the basis of American agriculture. Any corn consumed in the immature stage may be called sweet corn, but nearly all commercial sweet corn is based on one or more simple recessive alleles that alter the carbohydrate content of the endosperm.

Until 1961 the *sugary1* (*su1*) allele on chromosome 4 defined sweet corn. In 1961, John Laughnan of the University of Illinois released the first supersweet variety.[1] Currently, at least eight genes that affect carbohydrate synthesis in the endosperm are being used, either singly or in combination, in sweet corn varieties. Different endosperm types differ in eating quality, holding capacity (shelf life), and field emergence. They often require specialized seed production, planting techniques, and isolation from one another in the farmer's field to prevent cross-pollination. The new endosperm types have complicated seed and crop production, but they have increased quality of both fresh and processed sweet corn. This has increased consumer satisfaction, which in turn has benefited the producer.

While the primary difference between field and sweet corn is altered carbohydrate composition in the endosperm, sweet corn is distinguished from other types of corn by many genes that affect all phases of plant growth. These genes affect table or eating quality (flavor, tenderness, and texture), appearance of ears and plants, and seed viability.

Sweet corn is not a race of maize or a separate subspecies within the species *Zea mays* (L.).[2] It is differentiated from other types of corn by a gene or genes that alter endosperm starch synthesis and its use as a vegetable. Differences between sweet corn and field corn and literature specific to sweet corn will be reviewed. However, much of the literature on field corn is applicable to sweet corn and should be examined for a complete view of the crop.

II. ORIGIN AND HISTORY

A. Pre-Columbian Sweet Corns

Sweet corn having *su1* endosperm existed in Central and South America in the pre-Columbian period[3–5]. Indigenous sweet corns of South America belong to the Chullpi complex.[2] *Chullpi* is

Quechua for sweet corn. The center of dispersal of Chullpi is the Sierra of southern Peru, where it is grown at altitudes of 2400 to 3400 m.[5] Chullpi is also found in Chile and Argentina, and derived forms are found in Ecuador and Bolivia where they are named Chulpi and Chuspillo, respectively.[2,6,7] In its purest form, Chullpi has short, very wide, ovoid ears with 20 to 30 kernel rows.[5]

A second pre-Colombian race of sweet corn is Maiz Dulce of Mexico.[4] Maiz Dulce is centered in the state of Jalisco and grown mainly at elevations of 1000 to 1500 m. Kelly and Anderson believe that Maiz Dulce is related to Chullpi.[8] Wellhausen et al.[4] agree, stating that there is no known maize in Mexico from which it could have been derived through mutation, and that its large cobbed, many rowed ear resembles the sweet corns of South America. Ducillo del Noroeste, a sweet corn quite unlike Chullpi, is found in the state of Sonora in northwest Mexico.[4] It has longer, more slender ears with more regular kernel rowing than either Maize Dulce or Chullpi. Unlike other Latin American sweet corns Ducillo del Noroeste is adapted to lowland, arid, subtropical conditions. Ducillo del Noroeste may have originated from a hybridization between Maize Dulce and Reventador,[4] a popcorn grown in the same regions as both Maize Dulce and Ducillo del Noroeste.[4]

Despite its occurrence throughout Latin America, sweet corn was not widely grown, nor was it used for roasting ears or green corn. Sweet corn was considered too gummy when consumed in this fashion, and nonsugary varieties were used as green corn. Sweet corn was highly prized for other uses, including *pinole*, a confection prepared using a flour from dried *su1* grain; *kancha*, parched or roasted dry grain; and *chicha*, an alcoholic beverage.[5,9–11]

Sweet corn was grown in many areas of what is now the U.S. prior to the arrival of Europeans.[9–12] Erwin disputes this,[13,14] although he identified an archeological ear dated at 1200 to 1300 A.D. from New Mexico as sweet corn.[15] Data, mainly from anthropological studies, indicate that many of the peoples of what is now the southwestern U.S. maintained sweet corn, including the Pima and Papagos, the Hopi, the Zuni, and the Pueblos.[9] Will and Hyde[12] have documented widespread use of sweet corn by the peoples of the upper Missouri, and Carter[9] suggests that the Iroquois of the northeastern U.S. cultivated sweet corn. However, the earliest of these anthropological studies took place at least 50 years after sweet corn was reported in New England, time enough for sweet corn to filter back to the Indians. Based on the use of what is clearly sweet corn in the agriculture of many of these peoples, it seems that North American Indians were growing sweet corn in pre-Columbian times. A Hopi woman, when asked about the origin of sweet corn, replied that it had no origin as it has always been in existence.[10] Similar to South Americans, Indians of North America, with the possible exception of the Hopis, did not use sweet corn as roasting ears, but instead used it to make confections.[9,12,16]

B. Origin of Modern Sweet Corns

The origin of North American sweet corn, specifically the relationship between modern commercial types and Latin American sweet corns, is not clear.[4] There are two main theories regarding its origin. The first proposes that modern sweet corn is descended from Maiz Dulce and Chullpi, while the second suggests that North American sweet corn is of recent origin, resulting from a mutation to *su1* in field corn.

Erwin is the strongest proponent of the independent origin,[13–15] basing his conclusions on three main points: (1) lack of sweet corn in archeological collections, (2) observation of spontaneous mutations to the *su1* allele in field corn, and (3) the fact there was no written record of sweet corn in the U.S. until the nineteenth century. Erwin,[17] however, identified a pre-Columbian ear of sweet corn in North America, but he regards this as unrelated to the development of sweet corn in New England and is concerned by the lack of sweet corn in collections from northeastern Indians.[13] Because sweet corn was first described over 200 years after English settlers arrived, Erwin[14] concludes that sweet corn is most likely the result of a relatively recent mutation.

Manglesdorf[11] and Galinat[18] support the theory that North American sweet corns are descended from Maiz Dulce. Common descent of all sweet corns is indicated by morphological as well as

agronomic data.[11,18] Sweet corns of Indians of the upper Missouri have higher row numbers and thicker cobs than do their starchy counterparts.[12] Manglesdorf[11] considers this to be consistent with the hypothesis that North American sweet corn is related to the high row number, thick cobbed Central and South American sweet corns. A second line of evidence relates to the near lethality of the *su1* allele in many genetic backgrounds. Backcrossing *su1* into nonsweet backgrounds often results in nonviable seed. It is difficult to fix spontaneous *su1* mutants due to the poor seed viability.[11] In addition, the expression of many of the endosperm mutant genes is suppressed by the sweet corn background.[19] Thus, the successful development of a new *su1* variety based on a spontaneous mutation requires not only the mutation to *su1*, but also the accumulation of modifying genes that increase viability. The first reports of sweet corn in the northeast indicate that it was used for roasting ears rather than a confection. Galinat[18] suggests that the change in utilization to roasting ears may be due to the loss of a floury gene, which gives the undesirable gummy consistency found in sweet corns from Peru to Montana.[4,12]

The first written reference to corn that is indisputably sweet corn, based on the shriveled seed rather than simply corn eaten green, was in Thomas Jefferson's *Garden Book of 1810*.[14] There are reports of earlier sightings, but the written description occurs later than 1810. In 1822, a report by "Plymotheus"[20] was published in the New England Farmer outlining the first introduction of sweet corn to New England in 1779. He reported that a sweet corn, called Papoon corn, was brought to New England by a Captain Bagnal, who collected it while a member of the forces sent to destroy the Iroquois' food supplies. The validity of this report has been the subject of some debate,[9,14] and the account has assumed the status of a legend. Regardless of priority, it is clear from the written record that true sweet corn was not important, if known, among European settlers prior to the early 1800s. Soon after this time, interest in and improvement of the crop began. References to sweet corn and new varieties began to appear in garden magazines and seed catalogs.[9,21] The first new varieties were developed largely by crossing sweet corn with adapted starchy types.

C. Sweet Corn in the United States

'Darlings Early' is seemingly the first sweet corn to carry a proper name.[22,23] Released in 1844, it was derived from a cross between an early yellow flint and a white sweet corn. It was the precursor of many important sweet corn varieties.[18] 'Stowell's Evergreen', a high-row number variety, was the product of a cross between Menomony soft corn, presumably a Southern Dent, and northern sweet corn.[22] A third variety of considerable historical importance is 'Crosby'. Crosby was listed in catalogs by 1867 but its ancestry is unknown. Crosby differed from any of the types then known, having deep, tender kernels on 12 to 16 rowed ears.[22] It had unsurpassed quality and it became the standard of both the fresh market and canning industries in the northeast. Due to its wider adaptability and higher yields, Stowell's Evergreen was more important in the midwestern processing industry. Despite its importance and quality, it seems Crosby has contributed little to modern germplasm.

At least 63 sweet corn varieties were being offered by seed companies by 1899, most of which had white endosperm.[24] Yellow types were largely considered unfit for human consumption.[22] In 1902 'Golden Bantam' was released by the Burpee company. Golden Bantam had both excellent quality and wide adaptability, and rapidly dispelled the old prejudice against yellow corn. Yellow corn instead became the standard, and many of the popular white varieties were converted to yellow by crossing to Golden Bantam (Figure 6.1).[18,22] As a result, most of the yellow inbreds and hybrids today have some Golden Bantam in their pedigree.[24,25] Golden Bantam was derived from a cross of Darlings Early and an early Yellow Flint. Thus, it is at least 75% Northern Flint, and likely more, as the original white *su1* that went into Darlings Early must have had a higher level of Northern Flint in its background.[18] Morphologically, Golden Bantam is very similar to the eight-rowed Northern Flints.[26]

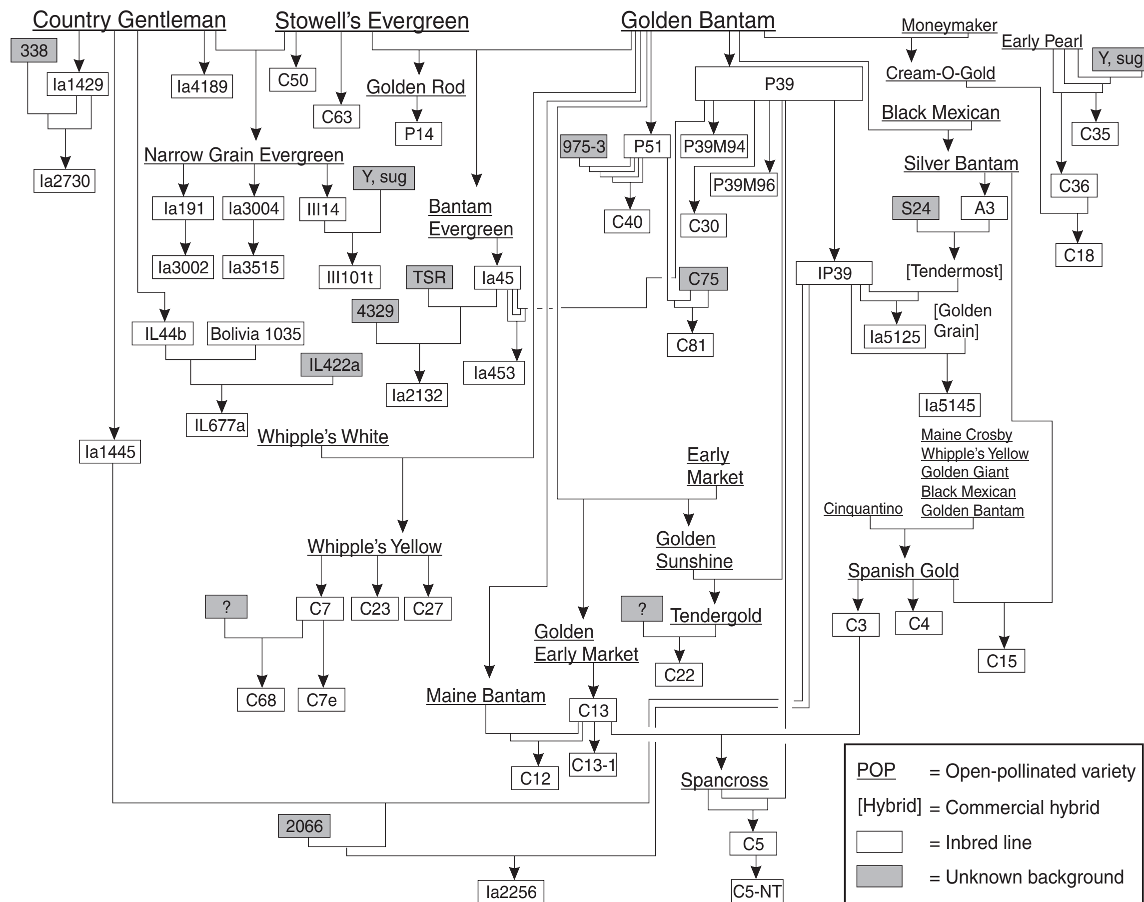


FIGURE 6.1 The phylogeny of some historically important sweet corn populations and inbreds that shows the influence of 'Golden Bantam' on yellow sweet corn inbreds. (Compiled by James T. Gerdes.)

Evidence suggests that modern sweet corn is related to the sweet corns of South America. Repeated outcrosses to local starchy varieties and selection for adaptation to local conditions must have occurred during its migration north. Because of the genetic bottleneck created by the importance of Golden Bantam and its descendants, the genetic base of modern sweet corn is largely Northern Flint.[26,27] Isozyme evidence, based on the average of 39 sweet corn inbreds, places sweet corn among the Northern Flint group, quite distinct from the Corn Belt Dent, Southern Dent, and popcorn lines that were all grouped fairly closely together.[28] At least 25% of these inbreds were of the Stowell's Evergreen or Country Gentleman types with no known Golden Bantam in their background.[29] Likewise, in a study with 60 U.S., 4 Mexican, and 2 Peruvian open-pollinated sweet corn varieties, Revilla and Tracy[27] found that all but four U.S. varieties clustered with the Northern Flints. The remaining four were clustered with Mexican and Peruvian corns, Southern Dents and Corn Belt Dents. Three of the four are known to be from Indians who live west of the Mississippi.[27] Northern Flint has attained a degree of genetic dissimilarity from other landraces, more similar to that of a separate species than of landraces of the same cultigen.[30] Northern Flint is no longer widely grown *per se* but is represented in a relatively pure form in some modern sweet corn varieties.

III. CURRENT ECONOMIC IMPORTANCE

Sweet corn is popular as both a fresh and processed vegetable. In 1996 the farm value of sweet corn for processing was approximately $259 million.[31] Among canned vegetables, sweet corn ranked second behind tomatoes in per capita consumption of canned product.[31] Among frozen produce, cut corn was second in pounds produced following potato products, while corn on the cob ranked forth.[31] Kaukis and Davis estimated the value added by processing increases the farm value by 300 to 400%.[32] In 1996, sweet corn grown for processing in the U.S. was 192,500 ha.[31] Approximately 55% was canned and 45% was frozen.[33] Yield of the processed crop increased from 10.1 Mg/ha in 1974 to 15.6 Mg/ha in 1996, while the value went from $54.30 per Mg to $86.50 per Mg.[31,34] In 1974 1.9 million Mg were produced for processing. By 1996, the total increased to 3.0 million Mg.[31] The increase in production was not offset by an increase in per capita consumption[34] or population growth[35] in the U.S. Excess production has been used to fuel a growing export market for processed product in Asia and Europe. In 1984 the U.S. exported 57,000 Mg of canned sweet corn and 33,000 Mg of frozen sweet corn. In 1996 the exports of canned and frozen product had risen to 177,000 Mg and 57,000 Mg, respectively.[36]

The north central states of Wisconsin, Minnesota, and Illinois account for 54% of the hectarage and 47% of the total tonnage, while the northwest states of Washington, Oregon, and Idaho account for 29% of the hectarage and 43% of the tonnage. The bulk of the remainder is produced in New York. Canada is second after the U.S. in sweet corn production. Sweet corn for processing is also grown in Japan, Southern Europe, Thailand, Australia, New Zealand, and parts of South America.

Sweet corn ranks sixth among fresh vegetables for per capita consumption.[31] In 1996 the farm value of sweet corn for fresh market was approximately $380 million.[31] This value was based on the 24 states included in the USDA statistics and, since sweet corn is grown in every state, the value was a low estimate. Total hectarage of the 24 reporting states was 89,600 ha in 1988. Florida leads the nation in fresh corn hectarage, with approximately 17,500 ha. Most of the Florida crop is shipped throughout the nation from October through May, with the spring crop being substantially larger than the fall crop. Georgia grows about 7000 ha, much of which is shipped north in late spring and early summer. Other leading states in fresh corn production are states that have large population centers such as California (9720 ha), New York (8500 ha), Pennsylvania (6800 ha), and Ohio (6000 ha) where corn is grown for local summer consumption. Average value of fresh corn climbed steadily from $7.80 per hundred weight in 1974, to $18.20 in 1995. Over the same time period yield per acre increased from 78 to 98 hundred weight and hectares produced increased from approximately 72,000 to 89,000.[31] Fresh sweet corn is popular in Canada, Japan, Taiwan, and Korea. Interest in sweet corn as a fresh vegetable is increasing in many other parts of the world.

IV. GENETIC BASIS OF SWEET CORN

When we eat sweet corn we consume immature kernels, consisting mainly of endosperm and ovary wall (immature pericarp). Genes that distinguish sweet corn from other types of corn affect these tissues. Sweet corn quality is determined by flavor, aroma, and texture of the endosperm and tenderness of the pericarp. Genes affecting ear and kernel appearance are also important.

A. Endosperm Mutants

1. Effects on Endosperm

Sweet corn flavor is determined in part by sweetness, which is affected by the amounts of sugar and starch in the endosperm. Due in part to its economic importance, starch synthesis in the corn endosperm has been the subject of extensive research. Comprehensive reviews covering starch biosynthesis and the genetic modification of endosperm carbohydrates have been published.[19,37–43] Most of mutants used in sweet corn improvement increase sugar content and decrease starch content. One mutant, *su1*, elevates the level of water soluble polysaccharide (WSP) (phytoglycogen) in addition to increasing sugar content.[19,41]

Many mutants of corn are known to affect endosperm development.[44] Fourteen have been used or studied for use in sweet corn,[19] and eight have been used commercially (Table 6.1). Most of these genes have been cloned and sequenced, and their specific enzymatic lesions are known (Table 6.1).[45–56]

Starch synthesis mutants may be divided into two classes based on their effects on endosperm composition.[19] The class 1 mutants, *brittle1* (*bt1*), *brittle2* (*bt2*), and *sh2* accumulate sugars at the

TABLE 6.1
Endosperm Genes that Have Been Used in Commercial Sweet Corn Cultivars

Gene	Gene symbol[a]	Chromosome[a]	Enzyme[a]	Phenotype[a]
amylose-extender1	*ae1*	5	Starch branching enzyme IIb	Glassy, tarnished, high amylose content
brittle1	*bt1*	5	Starch granule bound phospho-oligosaccharide synthase	Mature kernel collapsed, angular often translucent and brittle
brittle2	*bt2*	4	ADP-glucose pyrophosphorylase	Similar to *sh2* (below)
dull1	*du1*	10	Soluble starch synthase	Glassy, tarnished
shrunken2	*sh2*	3	ADP-glucose pyrophosphorylase	Inflated, transparent, sweet kernels collapse on drying becoming angular and brittle
sugary1	*su1*	4	Starch debranching isoamylase[b]	Wrinkled and translucent
sugary enhancer1	*se1*	2[c]	Unknown	Observed only in *su* lines; inflated, light colored, slow drying, color varies with background
waxy1	*wx1*	9	Starch-granule-bound ADP-glucosyl transferase	Opaque, endosperm stains red with iodine

[a] Coe et al.[44]
[b] Rahman et al.[52]
[c] Tadmor et al.[73]

TABLE 6.2
Carbohydrate Composition of Various Endosperm Genotypes at 21 or 22 Days after Pollination Or Mature Seed

Genotype	Dry weight (mg)	Starch (%)	Sucrose (%)	Reducing Sugars (%)	WSP[a] (%)	Dry weight (mature) (mg)	Starch (mature) (%)
			21 DAP[b]				
normal	68.5	80	2.9	4.8	—	—	—
brittle	55.2	16.9	19.3	21.3	—	—	—
brittle-2	38.9	7.4	17.6	41.6	—	—	—
shrunken-2	53.2	21.0	22.5	26.3	—	—	—
			22 DAP				
normal	87.0	48.9	7.3	3.9	0.2	246	83
amylose-extender	67.3	25.4	15.8	4.4	0.4	153	80
dull	72.7	42.1	11.8	4.3	0.3	142	76
shrunken-2	65.8	17.4	24.6	8.3	0.3	74	54
sugary	61.0	20.6	11.6	4.3	27.7	134	32
waxy	87.8	52.6	7.5	2.9	0.2	239	75

[a] Water soluble polysaccharides.
[b] Days after pollination.

Source: Adapted from Nelson, O. E., in *Advances in Cereal Science and Technology*, vol. 3, Pomeranz, Y. Ed., American Association of Cereal Chemists, St. Paul, MN, 1980.

expense of starch and have greatly decreased total carbohydrates at the mature seed stage (Table 6.2).[19,38] At 18 to 21 days after pollination (normal sweet corn harvest stage) these mutants have 4 to 8 times the total sugar found in nonmutant corn (Table 6.2).[38,41,57–63] Due to higher sugar levels, class 1 mutants may be used independently of other carbohydrate mutants in sweet corn varieties, and *sh2*, *bt1*, and *bt2* have all been used commercially. For processing, *sh2* types are currently the second most widely used endosperm type after *su1,* while for many fresh uses, usage of *sh2* has surpassed that of *su1*. Due to elevated sugar levels, varieties of these genotypes are often called supersweet or extra sweet.

Class 2 mutants, *amylose extender1* (*ae1*), *dull1* (*du1*), *su1*, and *waxy1* (*wx1*), alter the types and amounts of polysaccharides produced,[19] The alleles *ae1*, *du1*, and *wx1* generally result in slightly less starch in the mature kernel than nonmutant types (Table 6.2).[19,38,40,41] These three mutants result in smaller increases in total sugar content at 21 days after pollination relative to class 1 mutants (Table 6.2) and do not make acceptable sweet corn when used singly. However, complementary gene action of certain double and triple combinations of class 2 mutants results in sugar levels equal to those found in class 1 mutants (Table 6.3).[40,64,65] Commercial hybrids having the triple recessive genotype *ae1 du1 wx1* have been released.[66]

The *su1* allele, contrary to its name, does not have exceptionally higher levels of sugars, but instead results in greatly increased levels of WSP (Table 6.2).[38] Mature endosperm of nonmutant corn contains about 2% WSP, while endosperm homozygous for *su1* may contain up to 35% WSP.[40] Water soluble polysaccharide is a highly branched polysaccharide consisting of α-(1-4) glucans with α-(1-6) branch points.[39] Water soluble polysaccharide gives *su1* endosperm the smooth texture and creaminess, characteristic of traditional sweet corn varieties.[19,67–69] While high levels of phytoglycogen appear to be unique to *su1*, elevated levels are maintained when *su1* is combined with either *wx1*, *du1*, or *bt1*.[64,70–72] When *su1* is combined with *ae1, bt2*, or *sh2* phytoglycogen production is suppressed (Table 6.3).[64]

TABLE 6.3
Carbohydrate Content in the Endosperm of Five Corn Genotypes at Four Harvest Stages

Genotype	Kernel age	Total Sugars (%)	WSP[a] (%)	Starch (%)	Total Carbohydrates (%)
Normal	16	17.6	3.7	39.2	60.5
	20	5.9	2.8	66.2	74.9
	24	4.8	2.8	69.2	76.1
	28	3.0	2.2	73.4	78.6
su	16	25.7	14.3	23.3	65.3
	20	15.6	22.8	28.0	66.5
	24	13.1	28.5	29.2	70.8
	28	8.3	24.2	35.4	69.6
sh2	16	28.3	5.6	22.3	56.1
	20	34.8	4.4	18.4	57.6
	24	29.4	2.4	19.6	51.4
	28	25.7	5.1	21.9	52.8
su sh2	16	33.1	5.0	7.2	47.3
	20	33.5	4.9	11.7	50.1
	24	27.8	4.6	14.4	46.9
	28	24.5	4.9	15.7	45.4
ae du wx	16	46.7	4.2	15.9	66.7
	20	38.7	3.6	26.6	68.9
	24	34.3	4.5	31.1	69.9
	28	28.1	4.9	32.0	65.1
LSD(0.05)	—	10.9	10.4	14.2	15.3
Genotypes within ages					
Normal within ages	16	17.6	3.7	39.2	60.5
LSD (0.05) ages within genotypes	—	5.8	4.8	7.6	7.5

[a] Water soluble polysaccharides.

Source: Adapted from Creech, R. G., in *Adv. Agron.,* 20, 275, 1968.

The *sugary enhancer1* (*se1*) allele[73] does not fit neatly into either class of endosperm mutants and its biochemical mode of action is unclear. When in combination with homozygous *su1*, *se1* results in sugar levels near those of *sh2* and WSP levels similar to unmodified *su1*.[74–76] This results in a high quality, sweet, creamy endosperm. Effects of *se1* were originally observed in Ill677a, a line derived from a three-way cross (Bolivia 1035 × Illinois 44b) × Illinois 442a (Figure 6.1).[77] Bolivia 1035 is an interlocking flour corn, while the other two parents are sweet corn inbreds. Later traits characteristic of *se1*, elevated total sugar, lighter kernel color, and slow drydown were attributed to the effects of a single locus.[78–80] The *se1* allele is not completely recessive but rather exhibits a dosage response, one dose of *se1* has little effect on sugar levels relative to non-*se* (*Se1Se1Se1*), two doses double the sugar levels, and three doses triple the sugar levels.[81] The *se1* allele has been shown to slightly elevate sugar levels in stocks not carrying *su1*[82] and may have been selected in the Bolivian corn for improved flavor. Due to its highly desirable effects of increasing sugar levels while maintaining phytoglycogen, *se1* was quickly incorporated into many breeding programs and some high quality *se1* varieties are now available. However, progress in developing *se1* hybrids has been slower than expected. Limitations to its use include the difficulty in identifying its presence in some genetic backgrounds and that the pale yellow kernel color

associated with the gene is undesirable in many markets. Also, it seems that a number of recessive modifiers are required to attain high quality *su1 se1* hybrids.[1]

The endosperm mutants now in use have other important effects on eating quality. These changes are usually discussed relative to *su1*. In *su1* endosperm, sugars are converted to phytoglycogen both pre-harvest and post-harvest, resulting in a continuous decrease in sugar content from the maximum attained around 18 to 20 days after pollination (DAP). At 28 DAP sucrose levels have declined by 50% or more from prime eating stage (Table 6.3).[41] Thus, the duration of the harvest period is very restricted for *su1*. Class 1 mutants maintain higher levels of sucrose, and at 28 DAP still maintain twice as much sugar as *su1* at 21 DAP (Table 6.3), thus significantly extending the harvest period.[83,84] Other quality factors, such as pericarp tenderness, will continue to decline. Therefore, to obtain the highest quality product, class 1 mutants should be harvested at the optimal time, about 18 to 24 (DAP), depending upon rate of development.[32] Endosperm with *ae1 du1 wx1* has a higher level of sucrose at prime eating stage than does *su1* and loses sucrose at a slower rate. However, the rate of loss seems to be slightly more rapid than the class 1 mutants.[32,41,67] Endosperm with *su1 se1* also has higher sucrose levels at 18 to 20 DAP, but *su1 se1* loses sugars at a rate similar to *su1*.[75]

Similar changes occur post-harvest. When kept at room temperature, *su1* endosperm loses 50% or more of its sugar in 24 hours (Table 6.4).[19,85] Rate of sugar loss can be reduced by cooling harvested corn, but even when stored at 5 to 10°C, one to two thirds of the sucrose may be lost in *su1* over a 3-day period.[85,86] These changes are reflected in significant reductions in taste test scores.[86] Not only do class 1 mutants start out with significantly higher levels of sugars at harvest than does *su1*, but the decline in sugar levels is much slower, even without refrigeration. Garwood et al.[85] found that after 48 hours at 27°C, *sh2* endosperm had double the sucrose content of a freshly picked near-isogenic *su1* endosperm (Table 6.4). When *sh2* was stored at 4°C there was no change in sucrose levels after 96 hours.[85] Due to extended shelf life, hybrids based on class 1 mutants are better suited for long distance shipping, as well as the habits of modern consumers. As is the case for timing of harvest, other factors affecting quality, most notably moisture content, which affects texture and tenderness, will decline over time. Loss of moisture content can be slowed by refrigeration. In cold storage, *ae1 du1 wx1* endosperm loses sugar more rapidly than do class 1 mutants but less than *su1*.[85] *Sugary enhancer1* goes into storage with more sugar than *su1*, but like *su1*, they convert sugar to WSP even under refrigeration.[86]

2. Combining Endosperm Mutants

In addition to the gene combinations already mentioned, *ae1 du1 wx1*, and *su1 se1*, many other combinations of endosperm genes are possible and some have been used commercially. The most common type of gene combination is partial modification based on two recessive genes. In the seed that is sown, one gene is homozygous and the other is heterozygous. The heterozygous gene segregates on the ear produced for consumption. Thus, 25% of the kernels express both endosperm mutants. Kernels in which both mutations are expressed usually have a higher sugar content, which is detectable by consumers.[67] Such hybrids are made by crossing an inbred homozygous recessive at one locus (e.g., *su1*) and homozygous dominant at another (e.g., *Sh2*), with an inbred homozygous recessive for both *su1* and *sh2*. The F_1 from such a cross would have the genotype *su1su1 Sh2sh2*. After pollination 75% of the kernels on the ear produced by the F_1 plant would be *su1su1 Sh2-* and 25% would be *su1su1 sh2sh2*. When *su1* is homozygous, 75% of the kernels have higher levels of phytoglycogen.[67] Thus, 75% of the kernels from such a hybrid will have the creamy texture of *su1* corn and 25% will be supersweet. The *su1su1 Sh2sh2* and *su1su1 Se1se1* combinations are most common, although there are commercial hybrids in which the F_1 is *Su1su1 sh2sh2*.

Partial modification is used to combine improved sweetness and acceptable seed quality. Supersweet hybrids, in general, have poorer germination than do *su1* hybrids, and seed of supersweet hybrids is more difficult to produce. The seed of *su1su1 Sh2sh2* hybrids will be phenotypically

TABLE 6.4
Carbohydrate Content of Three Corn Genotypes after 0, 1, 2, and 4 Days of Storage at 4° and 27°C

Storage Conditions		Carbohydrate (% of dry weight)			
Time (hr)	Temp. (°C)	Reducing sugar	Sucrose	WSP[a]	Starch
		sugary			
0	—	6.0a	14.4a	32.6d	12.1a
24	4	5.0ab	10.5b	38.2bcd	7.9bc
48	4	4.8abcd	12.9a	37.0cd	6.9bc
96	4	4.9b	9.9b	42.7abc	6.0c
24	27	3.7c	5.7c	45.2a	7.3bc
48	27	3.6cd	4.6c	41.4abc	7.4bc
96	27	3.1d	2.4d	43.3ab	9.2b
		shrunken-2			
0	—	5.3d	36.5a	0.9a	2.9b
24	4	7.0abc	32.7b	0.7a	5.0ab
48	4	7.9a	30.6b	0.7a	5.9a
96	4	7.6ab	33.9ab	0.8a	5.6a
24	27	5.7cd	29.0b	0.8a	5.2a
48	27	6.0cd	28.2b	0.8a	8.3a
96	27	4.4d	13.5c	0.6a	9.7a
		ADX[b]			
0	—	7.0bc	24.8a	7.0a	23.3b
24	4	7.8ab	22.0a	1.2c	25.9ab
48	4	9.2a	26.0a	1.3bcd	21.7b
96	4	7.4abc	22.1a	1.7bd	30.0ab
24	27	6.4bc	16.7b	1.4cd	30.7ab
48	27	5.7c	12.9bc	1.6cd	34.6a
96	27	5.5c	11.1c	2.5b	31.4ab
		sugary			
0	—	6.0a	14.4a	32.6d	12.1a
24	4	5.0ab	10.5b	38.2bcd	7.9bc
48	4	4.8abcd	12.9a	37.0cd	6.9bc
96	4	4.9b	9.9b	42.7abc	6.0c
48	27	5.7c	12.9bc	1.6cd	34.6a
96	27	5.5c	11.1c	2.5b	31.4ab

[a] Water soluble polysaccharide.
[b] Amylose extender, dull, waxy.

Source: Adapted from Garwood, D. L., et al., in *J. Amer. Soc. Hort. Sci.*, 101, 400, 1976.

su1 with good germination. Improved germination is not justification for developing *Su1su1 sh2sh2* hybrids. In these hybrids the seed will be phenotypically *sh2* and have the same germination potential as seed of an unmodified *sh2* hybrid. The slightly elevated sugar level in 25% of the kernels is apparently enough justification for developing *Su1su1 sh2sh2* hybrids.

An additional rationale for the use of partially modified hybrids is as new endosperm types become available they can be more rapidly incorporated into commercial form. When a *su1* inbred is converted to *sh2*, *su1su1 sh2sh2* inbreds are a by-product of the backcrossing. If a good *su1su1 sh2sh2* inbred is isolated new hybrid combinations can be made with all of the *su1* inbreds in the

breeding program. This can be accomplished much more rapidly than incorporating the new gene into all older inbreds or developing many new inbreds homozygous for the gene or genes of interest. Thus, one might expect that partially modified hybrids will become less important as more inbreds containing the new mutants are developed.

Despite the advantages of these partially modified types, there are a number of important problems. While the seed parent of a *su1su1 Sh2sh2* hybrid is relatively easy to produce, the male parent is usually double recessive, *su1su1 sh2sh2*, which, typically, has poor germination. If cold soils or other adverse conditions are present at planting time, the male parent might not emerge, and with no pollinator present, the seed crop would be lost. This problem would not be as serious if the pollen parent was an *su1su1 se1se1* type because of the relatively better germination compared with *su1su1 sh2sh2*. A second problem for all partially modified hybrids can occur during processing because the raw product is not uniform (two types of kernels on the ear). Kernels with different sugar content may respond differently to processing. This is especially a problem with whole ear frozen corn, in which the high sugar kernels may appear brownish or tan next to the normal yellow of the low sugar kernels. Processors may avoid the problem by adjusting blanching temperature, and should be aware that they are working with a nonuniform raw product.

Combinations in which three recessive genes are used, leading to partial modification, have been developed.[87,88] When in the F_1 two genes are heterozygous and one is homozygous, 44% (7/16) of the F_2 kernels have elevated sugar levels. The most common trigenic combination is probably *su1su1 se1se1 Sh2Sh2* X *su1su1 Se1Se1 sh2sh2*.[88]

The genotype *su1su1 se1se1* is an example of 100% modification in which both parents are homozygous for two recessive alleles.[67] The *su1su1 se1se1,* commonly called sugary enhancer or sugary enhanced, is probably either the most or second most widely grown endosperm type for local fresh market production.

Other combinations resulting in 100% modification have been proposed.[72,89] While *su1 sh2* hybrids have been developed that will germinate at acceptable levels under ideal conditions, it is unclear whether these hybrids will ever gain commercial acceptance due to difficulty in seed production and unreliability in field emergence under stressful conditions.

Galinat[89] developed a method using the *Su1* allele on *Tripsacum* chromosome 7 to mask the effect of the *su1* allele in *su1su1 sh2sh2* × *su1su1 sh2sh2* crosses so that the seed the producer sows is phenotypically *Su1 sh2*. In the F_1, the addition *Tripsacum* chromosome has only about a 10% transmission rate, because of the lack of a homologous chromosome with which to pair during meiosis. Thus, approximately 90% of the kernels on the ear to be consumed are *su1su1 sh2sh2*. The cytological expertise required to detect and maintain addition *Tripsacum* chromosomes is not available in most seed companies and this method has not been used commercially.

High-sugar mutants generally have reduced field emergence and seedling vigor. Acceptance of *sh2* varieties was delayed due to these problems as well as differences in texture and tenderness. Significant improvements have been made in all these parameters and high-sugar corns have gained major shares of the market. In some cases, such as the Florida long-distance shipping market and Japan, *sh2* varieties are the predominant type.

3. Effects of Genetic Background on the Expression of Endosperm Mutants

While single genes, such as *su1* and *sh2*, have major effects on carbohydrate composition in the endosperm, genetic background also has major effect on carbohydrate composition. Soberalske and Andrew[84,90] detected significant endosperm by inbred interactions affecting moisture, sugar, WSP, and starch levels among seven inbreds near-isogenic for 10 different endosperm mutants or combinations of mutants. Total sugars among *sh2* versions of the inbreds ranged from 38 to 46%, while sugar levels in *su1* versions ranged from 13 to 26%.[84] Wong et al.[91] examined chemical components of quality of 24 commercial *sh2* hybrids and found extensive variation for sucrose, total sugar levels, and other traits. They concluded that the variation was due to allelic variation at loci other than *sh2*.

Results of a diallel cross involving seven *su1* inbreds, indicated significant general combining ability (GCA) effects for accumulation of sucrose and WSP, whereas specific combining ability (SCA) effects were not significant.[92] In contrast, Michaels and Andrew[93] observed significant SCA effects for sucrose accumulation in a diallel study with five *sh2* inbreds, but GCA effects were not significant. Whether these differences are due to different endosperm mutants used in the two studies or different inbred parents is unresolved. Michaels and Andrew[93] did observe significant GCA and SCA effects for the accumulation of total sugars in the *sh2* diallel. Sweetness, as judged by a taste panel, showed significant GCA and SCA effects in a seven-line *sh2* diallel.[94] Inbred background also has significant effects on maturity related changes in WSP, sugar, and moisture levels.[95] Significant maternal effects on sugar composition and moisture content have been detected.[95] The nature of the maternal effect is unknown.

The importance of genetic background or modifying genes on carbohydrate composition is also indicated by the effectiveness of recurrent selection programs aimed at altering endosperm composition within an endosperm type.[96,97] Juvik et al.[96] observed an increase in starch levels from 17 mg/kernel to 27 mg/kernel over 10 cycles of selection in a *sh2* population. Selection criteria in the recurrent selection program were seed weight and field emergence.[98] Chang[97] demonstrated the effectiveness of visual selection in changing the proportion of pseudostarchy kernels in a *su1* population. Pseudostarchiness is related to relative amounts of sugar and starch in the endosperm.[99] Thus, not only is carbohydrate composition affected by modifying factors, it is also easily altered through selection.

A powerful indication of the importance of background effects on the phenotype of endosperm mutants is the lethality or near-lethality of the *su1* gene when it is backcrossed into field corn lines. While sweet corn lines homozygous for this allele often have nearly 100% germination, *su1* can only be maintained as heterozygote in many field corn backgrounds.[100]

4. Allelic Variation in Endosperm Mutants

Many important endosperm genes, including *su1*, *sh2*, *bt1*, and *bt2*, have extensive allelic series.[19,44,72] Phenotypic expression of these alleles ranges from kernels that are smooth seeded and normal appearing to kernels so defective as to be lethal. The presence of some normal appearing alleles can only be detected when another gene is present. Such is the case of *sugary1-amylaceous* (*su1*-am) and *sugary1-66* (*su1*-66), which are revealed in combination with *du1*.[44] It is not known if all the hybrids of a given endosperm type have the same allele at that locus. However, it has been suggested that certain alleles of *sh2* and *bt2* may have better seed quality, while maintaining adequate levels of sugar for acceptable flavor.[42,72]

B. Other Quality Traits

1. Aroma and Tenderness

Sweetness is the major component of flavor in sweet corn.[101,102] Flavor is also determined by aromas, especially those released during cooking. In a headspace analysis of cooked corn, Flora and Wiley[101] found seven compounds that produced aromas, one of which, dimethyl sulfide, was described as corny (Table 6.5). Many of these compounds are repulsive in their pure state, but in combination produce a pleasant aroma.[101] Dimethyl sulfide seems to be the dominant compound determining the aroma of cooked corn. Other aromas detected were described as fruity.[101] Higher levels of these compounds may be responsible for off-flavors noted in progeny of some crosses between sweet corn and non-sweet germplasm. Genetic control of these compounds is unknown, but varieties vary in amounts of dimethyl sulfide and hydrogen sulfide.[103,104] Flavors and aroma can be selected by taste testing during breeding.

Tenderness is determined by the resistance of the pericarp to chewing,[24] and is negatively correlated with pericarp thickness.[105,106] The number of genes determining pericarp thickness is

TABLE 6.5
Chemical Identities and Aromas of Compounds Detected in Headspace Analysis of Cooked Corn

Compound	Aroma
Hydrogen sulfide	Rotten egg
Methanethiol	Unpleasant, sulfurous, fecal
Acetaldehyde	Fruity
Ethanol	Slightly fruity, ethanolic
Ethanethiol	Sulfurous
Dimethyl sulfide	Corny
Unknown	Musty, grainy

Source: Flora, L. F. and Wiley, R. C., in *J. Food Sci.*, 39, 770, 1974.

unknown, although in some crosses a major gene appears to condition thinness with modification for thickness by a number of minor genes.[107,108] Pericarp thickness of an F_1 of a thin by thick cross tends to be thinner than the midparent value.[107] Estimates of the heritability of pericarp thickness are relatively high, 80%, and thickness can be altered by recurrent selection.[109] Ito and Brewbaker[106] reported a decrease in pericarp thickness from 74 microns in cycle 0 (C0) to 53 microns in C3, or 6.8 microns per cycle, in a mass selection program. Pericarp is the outermost layer of the corn kernel and is derived from maternal tissue. However, pericarp thickness is affected by the endosperm type.[110–112] The effect of the endosperm type varies according to inbred background, but in some instances it is large enough to affect tenderness.[111]

C. Genetics of Appearance and Performance Traits

Sweet corn differs from field corn by a number of important traits affecting the appearance of both the husked and unhusked ear, in addition to the those affecting palatability. These traits vary in importance depending upon the intended end use of the hybrid (Table 6.6).[32] Our knowledge of the genetic control of most of these traits is incomplete.

1. Cob and Kernel Color

White cobs and clear pericarp are important sweet corn characteristics and most sweet corn is homozygous for the allele *P1-ww* at the pericarp-cob color locus. Sweet corn lines often carry the *C1-I* allele, which inhibits anthocyanin development in the aleurone[44] and most *sh2* varieties carry the *a1* allele, which inhibits anthocyanin development in the entire plant and is tightly linked to *sh2*. The aluerone is also colorless in nearly all commercial sweet corn varieties, but some open-pollinated

TABLE 6.6
Traits Important in Sweet Corn Cultivars and Their Relative Importance in the Fresh Market and Processing Market

Trait	Description	Unit	Market			
	Yield		F[a]	Wk[b]	CS[c]	FC[d]
Green weight	Weight of sample in husk	Kg	1[g]	3	3	2
Yellow weight	Weight of husked ears	Kg	e	2	2	2
Cut corn weight	Cut but unwashed	Kg	1	3	3	1
Recovery	Percentage of cut corn of green weight	%	1	3	3	1
Number of ears	Ears per plot	no.	3	2	2	3

TABLE 6.6 (CONTINUED)
Traits Important in Sweet Corn Cultivars and Their Relative Importance in the Fresh Market and Processing Market

Trait	Description	Unit	Market			
Weight of ear	Average of usable ears	Kg	e	1	1	1
Length of ear	Average	cm	e	2	2	3
Diameter of ear	Average	cm	e	2	2	3
	Ear appearance					
Row number	Modal number and range	no.	3	2	2	3
Silk color	White or clear preferred	rating	3	2	2	3
Tip fill	Measurement of distance from tip where usable kernels begin	cm or rating	3	2	2	2
Row configuration	Straightness of rows	rating	3	3	2	3
Ear shape	Tapered vs. cylindrical, curvature undesirable	rating	f	f	f	f
Ear uniformity		rating	3	3	2	3
Kernel appearance						
Color	Brightness and shade	rating	2	3	3	3
Depth		mm	1	3	2	2
Width		mm	2	3	1	2
	Kernel quality					
Flavor	Sweetness and corn flavors	rating	3	3	2	3
Texture	Creamy vs. watery vs. starchy	rating	3	3	2	3
Tenderness		rating	3	3	2	2
	Plant characters					
Tassel date	50% of the plants shedding pollen	date	f	f	f	f
Silk date	50% of the plants silking	date	f	f	f	f
Ease of snap	Ease of ear removal by hand	rating	2	1	1	1
Plant uniformity		rating	2	3	3	3
Plant habit	General plant appearance	rating	f	f	f	f
Root lodging	Percentage of plants lodged at the root	%	f	f	f	f
Stalk lodging	Percentage of plants with broken stalks	%	f	f	f	f
Tiller number		no.	f	f	f	f
Husk appearance	Color; dark glossy to pale green	rating	3	1	1	1
Husk protection	Length of husks beyond ear tip	cm	3	2	2	2
Flag leaf length		cm	3	1	1	1
Huskability	Ease of machine husking	rating	1	3	3	3
Plant height		cm	e	2	2	2
Ear height		cm	e	2	2	2
Seedling vigor		cm	f	f	f	f
Seedling uniformity		rating	f	f	f	f
Pest resistance	Rating of resistance to important insects or pathogens	% or rating	f	f	f	f

[a] Fresh market.
[b] Whole-kernel processing.
[c] Cream-style.
[d] Whole ear frozen.
[e] Importance varies according to maturity, region, or other factors.
[f] Of equal importance in all markets.
[g] 1 = relatively unimportant, 3 = important.

varieties such as ‘Black Mexican’ and ‘Sweet Baby Blue’ have purple aluerone.[26] These types of varieties usually have white kernels at the eating stage and purple pigment begins to accumulate after prime eating quality is passed. Recently seed companies have released sweet corn hybrids that have colored kernels at the prime eating stage. ‘Ruby Queen’ from Burpee has red pericarp and ‘Indian Summer’ from Harris has purple aluerone. It is too early to speculate on consumer acceptance of these new types

Endosperm color is also important with three main types of varieties: yellow, white, and bicolor (25% white and 75% yellow on the same ear). Yellow corn is the most important for processing although white corn is also processed. Yellow corn is predominant in the local fresh and long distance shipping markets although regional preferences vary, with bicolors being particularly important in the Northeast and white endosperm hybrids from the mid-Atlantic region through the south. Bicolors have become important in the U.S. midwest fresh market and Japan. Yellow (*Y1*) vs. white (*y1*) endosperm is controlled by the *Y1* locus with white being recessive to yellow. Bicolor hybrids are produced by crossing a yellow inbred, *Y1Y1*, and a white inbred, *y1y1*. When the resulting F_1, *Y1y1*, pollinates in the grower’s field a segregation of three yellow to one white kernel will appear on the ear. Although *Y1* is required for yellow endosperm, modifying genes have an effect on the shade of yellow, with a bright glossy yellow being the desired type.

The first commercial bicolor ‘Sugar and Gold’ was developed in the late 1940s by Oscar Pearson a breeder with Eastern States Farmers Exchange in western Massachusetts. Sugar and Gold was followed very quickly by another bicolor developed by Pearson, ‘Butter and Sugar’ that set the standard for bicolor varieties for many years.[113]

2. Genetics of Ear Appearance Traits

Since sweet corn is often consumed directly on the ear, many traits that are of relatively little importance in other types of corn are of critical importance for fresh market or whole ear frozen corn. Ear appearance traits include number of kernel rows, row configuration (straightness and arrangement), tipfill, kernel width and depth, ear shape, and ear size. Standards for these characteristics vary from market to market and over the course of a growing season, with early-maturing varieties usually permitted more flaws than main-season varieties. Adequate husk extension is important in reducing bird and insect damage. In many areas the appearance of the husks on fresh market sweet corn is important, with long, dark green flag leaves being preferred. In most U.S. markets, the preferred fresh market type would have at least 16 straight rows of small, deep kernels, with the kernels filled to the tip of a 20 to 23 cm ear.

The most important traits for whole-kernel processing are those that affect kernel appearance and cut kernel recovery, such as kernel color, width, and depth. Deeper kernels result in more recovery, or usable yield, from the same tonnage of corn than do shallow kernels. Husks of processing varieties must be relatively easy to remove by mechanical huskers, without damaging the tender kernels. Ear shape is important in processing, as a slightly tapered ear helps automated kernel cutters orient the ears for maximum efficiency. White or light green silk color is required in both fresh market and processed corn.

The few studies investigating inheritance of these traits in sweet corn indicate that most of these traits exhibit polygenic inheritance, usually with significant genotype by environment effects.[24,100,114,115] Hansen et al.[114] studied inheritance of tassel date, silk date, plant height, ear height, shank length, husk extension, tipfill, row number, ear length, and first ear weight in a diallel involving seven sweet corn inbreds. They found that both GCA and SCA effects were significant for all traits. The ratio of SCA variance to GCA variance was greater than one for two traits, ear length and ear width.[114] Heritability estimates based on variance components ranged from 40% for ear weight to 88% for row number. Reciprocal differences were detected for all traits except ear weight.[116] In some crosses, the differences between reciprocals were large enough to be of economic importance.[116] In a similar study, Tracy[100] examined the

inheritance of ear weight, yield per hectare, total ears per plant, usable ears per plant, plant and ear height, tillers per plant, ear diameter and length, silk date, ear shape, tipfill, and row configuration using a diallel of seven inbreds. GCA effects were significant for all traits, and SCA effects were significant for all traits except ear height, tiller number, and ear shape. No reciprocal differences were detected for any traits.

3. Zig-Zag or Country Gentleman Row Arrangement

Row configuration is inherited polygenically, but a special case exists. 'Country Gentleman' type kernel arrangement, also called shoepeg or zig-zag, characteristically has no obvious rows. Instead, kernels appear to be arranged on the ear in a random fashion. In corn, there are two florets in each spikelet on the ear, and in most germplasm only the upper floret develops into a functional flower.[117] However, in Country Gentleman corn both the upper and lower florets develop resulting in twice as many kernels on an ear relative to normal corn. Crowding of the kernels results in the loss of regular rowing. Huelson and Gillis[118] found that Country Gentleman kernel arrangement is determined by two genes, and that straight row arrangement is incompletely dominant over Country Gentleman row arrangement. Some crosses in their study, however, did not fit a two-gene model, and it seems that inheritance of this type of rowing is more complex in many crosses. Varieties with Country Gentleman type rowing are still sought after in a small portion of both fresh market and processing industries.

V. GERMPLASM RESOURCES FOR SWEET CORN IMPROVEMENT

A. Historical Perspective

Tapley et al.[22] listed approximately 850 open-pollinated sweet corn varieties. Many of these were synonyms and the total number of different varieties was approximately 300. Contributions of these varieties to modern germplasm is highly variable with Golden Bantam and its derivatives having the greatest contribution followed by Evergreen and Country Gentleman.[16] These three varieties were the initial source material for inbreeding at the state experiment stations. Programs interested in yellow fresh market corns emphasized Golden Bantam types, while those concentrating on processing corn extracted inbreds from Evergreens and Country Gentleman.[119–124]

The single-cross hybrid, Golden Cross Bantam, was released in 1933.[119] Golden Cross Bantam rapidly became the most popular hybrid and the standard for judging other hybrids, due to its high quality, resistance to Stewart's wilt (*Erwinia stewartii* E. F. Smith), and yield.[16] The parents of Golden Cross Bantam were derived from different strains of Golden Bantam, P39 from an improved high row-number strain and P51 from an eight-rowed strain.[119] Both of these inbreds, but especially P39, were widely used as source material for development of new inbreds (Figure 6.1).[16]

Similar to field corn breeders, sweet corn breeders rapidly moved away from selfing out of open-pollinated varieties after the first cycle of inbred development, and instead concentrated on deriving new inbreds from crosses between elite inbreds. Inbreds were exchanged among experiment stations and the emphasis on specific open-pollinated varieties declined. Golden Bantam derivatives were crossed to Evergreen materials forming the basis of much of the publicly released inbred germplasm for processing types (Figure 6.1).[16] Leaders during this era were W. R. Singleton and D. F. Jones at Connecticut, G. M. Smith at Purdue, E. S. Haber at Iowa State, and W. A. Huelson at Illinois. A number of inbreds released between 1930 and 1960, or their *sh2* conversions, are still important in commercial hybrids today. Nearly all yellow endosperm inbreds have some Golden Bantam in their background (Figure 6.1).[16,26,27] The contributions to modern germplasm of the Connecticut, Illinois, Iowa, and Purdue breeding programs are well documented.[16,32,125,126]

B. Current Resources

1. Adapted Commercial Sweet Corn

Importance of public inbred and hybrid development programs, relative to private programs, has declined since the 1950s. In a 1982 survey by Kaukis and Davis,[32] there were 21 professionals working on sweet corn breeding in 11 private companies and 5 FTEs (full time equivalents) at 10 public institutions. In 1999, two universities had active hybrid development programs, while three other states had varying levels of commitment to inbred and population development and germplasm enhancement. With this change in emphasis from public to private, it is difficult to determine the germplasm contributing to current varieties. However, the main breeding resources in sweet corn, as in field corn,[127] are elite hybrids and inbreds, which are recombined and selected for improved performance. The hybrid 'Jubilee' deserves special mention in this regard. Released around 1960, it quickly became the predominant hybrid in the processing industry, and undoubtedly has been used as source material in many breeding programs. Jubilee is a proprietary hybrid and the backgrounds of its parents are not publicly available.

Varying levels of resistance to many pests are available in adapted sweet corn. Partial resistance to common rust (*Puccinia sorghi* Schw.) is available within elite sweet corn germplasm.[128–130] The level of partial resistance in some currently available hybrids is high enough to prevent economic damage under most conditions.[131] Adequate levels of resistance to Stewart's wilt, northern corn leaf blight (*Exserohilum turcicum* Pass.), Goss's wilt (*Clavibacter michiganense* ssp. *nebraskense* Schuster, Hoff, Mandel, and Lazar), common smut (*Ustilago maydis* DC.), and head smut (*Sphacelotheca reiliana* Kuhn) exist within elite varieties.[129,132,133] For many of these diseases, extreme susceptibility exists within a limited part of the gene pool as is the case of Goss's wilt and certain field corn inbreds.[134] Avoidance of these highly susceptible types results in adequate resistance.

Lower levels of resistance to insect pests have been reported in adapted sweet corn germplasm. Sweet inbreds with some resistance to the corn earworm (*Heliothis zea* Boddie) have been reported,[135] and mass selection in populations based on these inbreds was successful in increasing resistance.[136] Based on this work, Wann[137] released a composite, 9E-79, that has both antibiotic and physical resistance to the corn earworm. Low levels of resistance to European corn borer (*Ostrinia nubilalis* Hubner) have been detected in sweet corn.[138,139] However, given the high pressure exerted by the corn borer, and the low levels of damage acceptable to the consumer or processor, the economic utility of these sources of resistance is unclear. A sweet corn population, AS9, with moderately higher levels of resistance to leaf feeding by the European corn borer has been developed.[140] Reduced leaf feeding however, has little effect on reducing economic losses due to ear feeding.[141]

2. Open-Pollinated Varieties

Utility of open-pollinated land races for developing new varieties depends in part on the level of advancement of modern commercial varieties. For the most part, inbreds used in current sweet corn hybrids are four or more breeding cycles removed from open-pollinated progenitors and highly refined for important commercial characteristics. It is unlikely that new inbreds selfed from open-pollinated varieties would be competitive with fourth or fifth cycle inbreds. However, open-pollinated varieties may have alleles useful for sweet corn improvement and fewer negative alleles than other sources of germplasm.

Hundreds of open-pollinated varieties were available at the beginning of the twentieth century, but only about 50 are presently available. The majority of the remaining varieties have been evaluated for germination and seedling growth under cold temperatures[142] and reaction to a number of important pathogens.[143] Hotchkiss et al.[142] evaluated the seedling cold tolerance of 35 open-pollinated varieties. They found substantial variation for the ability to grow under cold temperatures

among the varieties. A few of the varieties grew very well under cold temperatures equaling the performance of Mexican high altitude sweet corn and exceeding Corn Belt Dent germplasm that had been selected for cold tolerance.[142] Pataky et al.[143] screened 36 varieties for reaction to Stewart's wilt, common rust, northern leaf blight, and southern leaf blight (*Bipolaris maydis*). The resistance of modern commercial germplasm to all four diseases was greater than that of the open-pollinated varieties.[143] A few of the open-pollinated varieties were resistant to certain diseases and may represent unique sources of resistance alleles.[143]

3. Non-Sweet Germplasm

Some sweet corn breeders have resisted using of non-sweet germplasm, warning against the difficulties in retaining table quality factors and specific raw product characteristics important in sweet corn.[24,32,124] While it is difficult to document, clearly some commercial breeding programs frequently use non-sweet germplasm, while others avoid it. Non-sweet germplasm has been used to improve sweet corn since the beginning of sweet corn breeding, in some cases with dramatic success. Non-sweet germplasm may be a source of increased disease and pest resistance.

Initially, the only possibility, other than to select within the limited sweet germplasm, was to cross with non-sweet types. The development of Stowell's Evergreen, and Golden Bantam, along with many other examples, indicates the success of this strategy.[16] One of the last developed open-pollinated varieties for the temperate zone was released in 1931 by Jones and Singleton[144]; 'Spanish Gold' a new variety of yellow sweet corn. Spanish Gold was developed by crossing 'Cinquantino', an early flint corn from the Pyrenees region of northern Spain, with a number of sweet corn varieties including Golden Bantam. Spanish Gold became the source population from which the very early-maturing inbreds, C2A, C3, C4, and C5, were derived. Some of these inbreds, which were developed in the 1930s, are still used commercially today.[124] Galinat[18] states that Spanish Gold derivatives were important in extending the range of sweet corn hybrids into regions with cooler, shorter growing seasons. Today a group in northwest Spain is using cold tolerant Spanish non-sweet populations and inbreds to adapt sweet corn to the European Atlantic coast.[145,146]

Haber[147] was a strong proponent of using Corn Belt Dent inbreds in sweet corn improvement, arguing plant health and vigor could be improved by doing so. An inspection of the pedigrees of sweet corn inbreds released by Haber's program at Iowa State University reveals that a number of field corn lines were used and, in most cases, they constituted 25 to 50% of the background of new sweet corn inbreds.[148] One of the more important publicly developed inbreds is Ia2132, which is one of the parents of 'Iobelle'. A *sh2* conversion of Ia2132, Fla32, is a parent of 'Florida Staysweet'.[125,126] Currently, other *sh2* derivatives of Ia2132 are used in *sh2* hybrids. Twenty five percent of Ia2132 is derived from the inbred 'TSR', and while the origin of TSR is unknown, its designation fits the pattern of other field corn inbreds used by Haber. A phylogenetic study of sweet corn inbreds based on restriction fragment length polymorphisms grouped Ia2132 closer to field corn inbreds than other sweet corn inbred.[25] Haber[147] emphasized the importance of rigorous selection for table quality traits during inbreeding and warned that without it, quality could be lost when using field corn germplasm in sweet corn improvement.

A more recent example of the successful use of exotic germplasm in sweet corn is the discovery of *se1*. The *se1* allele was identified in IL677a, an inbred developed by A. M. Rhodes of the University of Illinois, which was derived from the cross (Bolivia 1035 X IL44b) X IL442a.[79] This inbred was the initial source for current *se1*. In addition, this inbred also exhibited a unique and useful type of resistance to common rust, which has been incorporated into many IL677a derivatives.[149]

While some resistance to many pests may be found within adapted sweet corn germplasm, higher levels of resistance are often available in non-sweet sources. Genes for resistance to common rust and maize dwarf mosaic virus from Corn Belt Dent germplasm have been incorporated into publicly released populations and inbreds and commercial hybrids.[150–152] Sweet corn

inbreds with partial resistance to northern corn leaf blight have been developed using Puerto Rican, Bolivian, and other tropical corn varieties.[153] Davis et al.[154] developed a rust resistant population using 11 *su1* inbreds and 17 Latin American varieties. The Corn Belt Dent inbred B52 was used as a source of genes for corn borer resistance,[150,155] and Latin American germplasm sources Maize Amargo, Zapalote Chico, and Antigua X San Juan have also been examined as sources of corn borer resistance.[156] However, given the very low tolerance of sweet corn consumers to corn borer damage and the complexity of resistance mechanisms, these efforts has not yet resulted in economic improvement.

In developing sweet corn for the tropics, Brewbaker has emphasized tropical germplasm sources, because most North American sweet corn germplasm lacks the pest resistance and stress tolerance needed to survive under tropical conditions with minimal inputs.[157–160]

Tracy[100,161] examined potential contributions and methods of incorporating both Corn Belt Dent and tropical germplasm in sweet corn improvement. Using a factorial mating design with four *su1* versions of field corn inbreds as males and seven sweet corn inbreds as females, Tracy[100] found that the field corn lines not only increased all vigor related traits such as yield, number of ears, ear weight, ear size, and plant height, but also improved ear appearance traits, such as ear shape, tipfill, and row configuration relative to sweet by sweet checks. Potential contributions of five Latin American populations adapted to temperate conditions were examined in a separate study.[161] The populations were NTZ Mexican Dent, NTZ Caribbean Flint, NTZ Cuzco, NTZ Cateto, and NTZ Coroico. Topcrosses with the Mexican Dent population had the best agronomic qualities for yield, root and stalk quality, and ear appearance traits. Those topcrosses having either the 'Coroico' or 'Cuzco' populations as parents were generally poor in all respects. It was suggested that 'Mexican Dent' and 'Caribbean Flint' populations have some utility in sweet corn improvement.

Davis et al.[162] developed two populations in an attempt to capitalize on the well-known Lancaster/Stiff Stalk heterotic pools of Corn Belt Dent.[163] NE-HY-13A was developed by crossing a sweet corn pool with seven Lancaster-type inbreds and NE-HY-13B resulted from crossing a different sweet corn pool with Stiff Stalk Synthetic/Reid-type inbreds. Davis et al. also developed the composite '1R' by crossing 11 sweet corn inbreds to 17 tropical parents.[164] The tropical parents were chosen because they all are consumed by humans as fresh (green) corn, and thus may have been selected for desirable flavor traits. Composite 1R was developed as a source of diversity for desirable traits for sweet corn improvement.

Sweet corn breeders choose breeding material from many sources, with elite sweet corn material being the first choice, followed by non-elite sweet corn germplasm, adapted non-sweet populations (Corn Belt Dent), and unadapted (tropical) populations. While Corn Belt Dent germplasm is a good source of genes for improved plant type, pest resistance, and stress resistance, many tropical corns are consumed directly by humans and have been selected for flavor and texture, unlike Corn Belt Dents. Therefore, in some instances, they may be a better choice for improving sweet corn.

4. *Tripsacum*

Rarely do breeders have the opportunity to reach to other genera for the improvement of a crop. Galinat[87] has proposed a method of producing "trisweet hybrids" using alien addition chromosome stocks from *Tripsacum*. Using Galinat's chromosome addition stocks sweet corn breeders can expand available germplasm resources to include *Tripsacum*.

5. Transgenics

Genetic transformation widens potential genetic resources to include all organisms. The *BT* gene from the bacteria *Bacillus thuringiensis* has been incorporated into commercial sweet corn hybrids.[165] The *BT* gene codes for a toxin that is effective in eliminating damage caused by certain families of insects. This offers excellent control of two of sweet corns greatest insect pests, the

European corn borer and the corn earworm.[165] Genes coding for resistance to certain viruses[166] and herbicides have also been incorporated into commercial sweet corn hybrids. Other resistance genes and quality factors will be incorporated into sweet corn varieties using transformation technology.

VI. BREEDING METHODS

A. Introduction

Sweet corn breeding, while using many of the techniques and theories developed by field corn breeders,[167] is very different in practice because of the different end use of the variety, the effect of xenia, and the highly perishable nature of the final product. These factors directly affect methods used in evaluating inbreds and hybrids. The generalized objective of any breeding program is to develop a variety that is better in some way than the one it is to replace and at least equal in all other respects.

Specific objectives, germplasm, and techniques depend upon the intended use for the new hybrid. For example, while yield is always important, the meaning of "yield" changes depending upon the market, with number of ears per hectare being important in whole ear frozen and fresh market corn, crates per hectare in long distance shipping corn, and tons per hectare and cases of cans per ton in canned corn. In certain markets weight of an individual ear may be important. Thus, yield data collected will vary with objectives and some methods of yield data collection require more resources than others.

Intended growing regions and regional preferences will also affect breeding objectives and techniques. Varieties intended for Florida or Asia must have *sh2* endosperm. However, most Florida corn has yellow endosperm, while Japanese markets prefer bicolor. Varieties bred for processing in the upper Midwest should have some level of rust resistance. But leaf diseases are not a problem in the Northwest, so a processing hybrid without rust resistance could be acceptable in that region. Despite the variation in regional requirements, the most successful hybrids are those that are adapted to greater range of environments and uses.

The target variety of nearly all sweet corn breeding programs will be a single cross hybrid; thus the breeder will develop inbreds, improved in some respect, to be used in making new hybrids. The value of an inbred can only be determined by its performance in hybrid combination. However, due to the relatively high heritability and additive nature of many traits important in sweet corn, selection during inbreeding is important. Selection for traits such as flavor, texture, tenderness, ear shape, row number and configuration, kernel shape and color, and husk appearance and protection can be effective during inbreeding. Inbred performance for traits strongly affected by heterosis, including yield parameters, must be determined in test crosses.

B. Methods

1. Pedigree Breeding

Pedigree breeding within backcrosses or single, three way, or double crosses as starting populations is the most commonly used method. Germplasm to be used as parents is determined by the breeding objectives. But in general, the most elite material available that has alleles for the traits of interest should be used. If the objective is to develop new better quality inbreds, a cross of two elite high quality individuals would be desirable, especially if one complemented deficiencies found in the other. If, however, the goal is to develop inbreds with good quality and with resistance to a disease that is not available in elite sweet corn, a cross of a high quality parent and a source of resistance, perhaps field corn or a more exotic source, might be required.

Selection for many traits can take place during selfing. Traits emphasized during inbreeding vary according to parental material and breeding objectives. In populations based on high quality elite inbreds, selection for highly heritable traits, such as ear appearance, plant habit, and maturity,

should be effective. If non-sweet germplasm is included in the parental material, taste-testing for acceptable tenderness and flavor should be performed at some point during inbreeding. Non-sweet germplasm often has relatively thick pericarp, although some thin sources are available.[157,168,169] Corn Belt Dent inbreds may impart off-flavors, and selection is required to eliminate these objectionable flavors. Tropical corns bred for direct human consumption may have more desirable flavors and thinner pericarp,[169] but testing is required to identify desirable segregates. Resistances to both biotic and abiotic stresses may be selected during inbreeding. Procedures for inoculation of most pathogens and insects attacking sweet corn have been developed and can be used during inbreeding.

Although many traits may be selected during inbreeding, combining ability of new lines must be evaluated. It is not clear at what generation of inbreeding most sweet corn breeders begin testcrossing. As is the case in field corn, early-generation (F_3, F_4) testing should be useful in estimating combining ability of new lines.[127] However, two factors in sweet corn suggest the advantages of testing in later generations (F_5, F_6). Uniformity is of even greater importance in sweet corn hybrids than it is in field corn. Hybrids made with early-generation lines are less uniform and more difficult to evaluate. Additionally, for many end uses of sweet corn, quality factors, which have relatively high heritabilities, are more important than yield. Many noncompetitive lines can be eliminated by adding one or two generations of inbreeding and selection prior to testcrossing.

2. Population Improvement

Development and improvement of populations, and their use as parental material, as is practiced in field corn,[167] is limited. A number of synthetic and composite populations have been developed by university and USDA researchers to be used by commercial breeders as germplasm sources. Many of these populations were developed as sources of pest resistance and are simply composites, having undergone little population improvement. However, a few have undergone intensive selection for adaptation and important sweet corn characters prior to release. In developing composite AS1R, Rubino and Davis performed ten cycles of 10% stratified mass selection for earliness, plant and ear appearance, and pest resistance.[164] Later, it was concluded that five cycles would have been adequate to gain the desired level of adaptation. However, even after ten generations of selection, significant genetic variability existed to permit continued improvement.[164] While mild selection for resistance to common rust under natural infection was practiced in this population, resistance decreased with selection for adaptation.[152] The populations MINN11, MINN14, and NECDR have undergone three cycles of full-sib recurrent selection for resistance to common rust with decreases in rust damage per cycle of 8, 12, and 13%, respectively.[170] MINN11 also responded to a divergent selection program for endosperm phenotype.[170,171] The selected types were extremely sugary and pseudostarchy. Not only did endosperm appearance change but also a number of desirable traits, such as kernel depth and narrowness and kernel-row number, improved due to indirect effects of selection.

Nearly all population improvement has as its objective creation of improved gene pools to be used as sources for development of new inbreds. However, Brewbaker has developed populations for direct use as open-pollinated varieties.[157–160] Brewbaker has used *sh2*, *bt1*, and *bt2* in different varieties, all of which are adapted to the tropics. Other than the work of Brewbaker, population improvement for the development of new varieties is limited in the U.S. to home gardeners and hobbyists.

3. Backcross Breeding

Backcross breeding is widely used in sweet corn improvement to transfer endosperm starch synthesis and color alleles into elite inbreds. It is also used to incorporate single gene disease resistances and transgenes. Typically, initial development of varieties based on new endosperm mutants has been backcrossing the new gene into elite *su1* inbreds, usually into both parents of an established cross. The *su1* allele is usually selected against during backcrossing. The parents of the first *sh2* hybrid, 'Illini Chief', and of the first *ae1 du1 wx1* hybrid, 'Pennfresh ADX', are *sh2* versions and *ae1 du1*

wx1 versions, respectively, of the *su1* parents of 'Iochief', Ia453 and Ia5125. Illini Chief had poor seed quality and a three-way cross was made (Ia453*sh2* × P39*sh2*) × Ia5125*sh2* and this became the first commercially successful *sh2* hybrid 'Illini Xtra Sweet'.[1] The parents of Florida Staysweet are *sh2* versions of the Ia2312 and Ia2256 parents of the *su1* hybrid Iobelle. The *sh2* versions of inbreds C13 and P39, originally released in the 1930s, are also used commercially today. Seed companies have used backcrossing to develop *sh2* and *se1* versions of successful *su1* hybrids such as Jubilee.

Backcrossing endosperm mutants into elite inbreds can be rapid and efficient. By selfing the backcross generation to detect the presence of the desired allele, and simultaneously crossing onto the recurrent parent, only one season per backcross cycle is required. If winter nurseries are available, three seasons per year are possible for most sweet corn inbreds. Multigene systems such as *ae1 du1 wx1* require more extensive testcrossing to ensure the presence of all desired alleles.

Expression of endosperm genes is affected by inbred background, and simple backcrossing with no selection may result in new lines with either poor germination or undesirable flavor. Therefore, selection should be performed during backcrossing to ensure acceptable performance of the new inbred.

The presence of *se1* maybe difficult to detect. In some backcrossing programs, the pale yellow kernel color associated with *se1* has been used as an indicator for the presence of the gene, although the lighter color is background dependent and, in general, undesirable. Other indicators used for *se1* are slower drydown rates, and more wrinkled kernels, both of which are also background dependent. Thus, incorporation of *se1* into commercial varieties via the backcross method has been difficult in some instances. Taste testing during inbreeding is the most reliable method of obtaining high quality *se1* inbreds.

4. Molecular Methods

It is possible to transform sweet corn, but due to the cost and difficulties associated with transformation most transgenes incorporated into sweet corn were first moved into field corn by transformation and then backcrossed into sweet corn. Due to undesirable factors affecting quality traits in field corn, direct transformation of sweet corn inbreds would be preferred. Presumably as transformation becomes more routine direct transformation of sweet corn inbreds will take place. The source of transgenes may be other organisms or corn genes that have been modified in some way.

In addition to genes affecting resistance to pests and herbicides, genes affecting quality traits will also be incorporated. Hannah et al.[42] proposed a method to improve germination of *sh2* corn, by attaching promoters that are active late in endosperm development to a functional *Sh2* allele. The new construct is transformed into *sh2* inbreds. Ideally, during seed production, the new *Sh2* gene would be expressed late in seed development and the resultant seed would have adequate starch and kernel fill to avoid *sh2* germination problems. In the farmer's field, ears for consumption would be harvested before the gene turns on and starch is produced. This method would probably reduce the harvest period for varieties containing such a gene.

The use of molecular markers for marker assisted selection programs has been suggested.[172,173] Maize is highly variable with an abundance of molecular polymorphisms. Genetic maps have been developed using a number of different markers systems and probes are readily available. In sweet corn RFLPs have been used to classify and evaluate the variation among publicly available sweet corn inbreds,[25] and to identify the chromosomal location of some quantitative trait loci (QTL).[174–176] Beavis,[177] however, has suggested standard QTL analysis techniques in corn underestimate the number of effective loci and overestimate the effects of the QTLs identified. RFLPs have been used to map single genes such as *se1*.[73]

Reports of the use of markers in breeding programs have been limited. Breeders at Pillsbury-Green Giant have used RFLPs in marker assisted recurrent selection to improve sweet corn populations (M. D. Edwards, pers. commun.). For some traits, gain was much more rapid using RFLPs, for others RFLPs offered no advantage.

5. Heterotic Patterns and Testers

Sweet corn does not have well-defined heterotic groups compared with U.S. field corn. There are a number of reasons for this, but perhaps most important is that yield is only one of a number of traits that are required for successful hybrids. Crosses among more divergent lines or populations will result in higher yields than crosses among related lines.[100,115] But wide crosses may also result in the loss of specific quality factors that are critical for success of the hybrid. Inheritance of many quality traits is additive or recessive. Thus, for maximum expression both parents need the same alleles, and the most likely way for this to happen is by developing new inbreds from the same high quality inbred. Often the highest quality lines in a breeding program will share a high proportion of their genome. Crossing such inbreds may be undesirable in terms of heterotic traits such as yield, but will allow the expression of recessive traits such as flavor.

Without well-defined heterotic groups, testers are chosen based on expected markets for the new hybrid and high general combining ability. In most programs testers are inbreds of proven commercial value. Because many important sweet corn traits are recessive, a tester that is recessive for these factors will expose the genotype of the new inbred and at the same time may result in a commercially acceptable hybrid.

6. Hybrid Evaluation

Regardless of method or germplasm sources used to develop new inbreds, efficient evaluation of their performance in hybrids is critical. As with inbred selection, specific traits and ways they are measured vary with the objectives of the breeding program. Compared with other types of corn, time span for evaluation of important yield and quality traits is very limited — only 5 to 7 days depending upon climate — limiting the number of plots that may be evaluated. Multiple planting dates may be used to increase the harvest season. However, in many regions length of planting season is limited by climate or pest pressures.

Initial screening of new hybrids is often based on a single harvest of small, unreplicated plots. Usually this initial screening will be done at one location, which often is not the intended area of use for the new hybrid. Selection of hybrids in the first stage is based on traits with relatively high heritability, such as ear and kernel appearance, ear and kernel size, and, if resources permit, flavor. Selected hybrids will be retested the following year.

Hybrids in second year tests usually are grown in replicated plots. At this stage plots may be harvested more than once to evaluate changes in quality parameters over time. Plots may consist of single rows, but multiple-row plots are preferred, as they reduce interplot competition and xenia effects.[178] Hybrids are evaluated for yield and/or yield components, such as kernel depth, ear size, and quality characteristics. Quality may be evaluated on raw or cooked product. If resources permit hybrids may be grown at a second location where they may be exposed to biotic or abiotic stresses. Also, during the second year of evaluation the breeder must ensure that enough seed of each hybrid exists for more extensive testing the following year.

During the third year of evaluation hybrids must be evaluated in replicated trials in the areas and under the conditions of commercial production. A number of locations usually are used with at least two planting dates. Unlike field corn, sweet corn is usually planted over a greater range of dates. For example, in Wisconsin sweet corn is planted from mid-April to the beginning of July. In Florida, normal planting dates span more than 6 months. Yield, table quality, harvestability, appearance, disease resistance, and other performance characters should be evaluated using methods appropriate to the specific target market over a number of harvests. When evaluating corn for processing, it is most desirable to have a small-scale processing operation in which hybrids can be canned or frozen. Not all breeders have such resources, and it may be necessary to evaluate processing qualities of a new hybrid in cooperation with a food processing company.

Because of the narrow harvest window for sweet corn hybrids, testing over multiple locations, planting dates, and harvests requires careful planning and timing. If the second location is more

than a few hours drive, a reliable cooperator should be identified or an outlying research station might be established. It is possible to evaluate general ear and plant appearance and pest resistance over a longer period of time, and outlying plots could be used for such data.

The final year of evaluation is devoted to gathering performance data under on-farm conditions or in specialized trials. Samples of hybrids may be sent to collaborators in other regions to gain information on adaptation. Specialized trials include management variables, such as plant density, nutrition, irrigation, or artificial epiphytotics of pests to which the new hybrids have not yet been exposed.

Selection among hybrids becomes more difficult and cost per hybrid for evaluation increases dramatically with each testing stage. Thus, rigorous culling of hybrids is important at each stage. Objectives of most programs include identification of elite hybrids that have stable performance over seasons and environments. It is highly unlikely that mediocre hybrids will improve with further testing. If an otherwise elite hybrid is seriously damaged by an environmental factor that it is likely to encounter in its area of intended use, it should be eliminated from consideration for that area. To continue evaluating such hybrids not only increases cost of trialing, but also takes space that could be used to evaluate new hybrids. Thus, rapid and final decisions will increase efficiency of testing.

Prospective processing hybrids should be canned or frozen, or both, during later stages of evaluation. Evaluation of processing characteristics greatly increases the cost and logistics of testing. Eliminating hybrids from consideration as processing types early in testing reduces these costs.

Most hybrids will be lacking in some character or other and compromises may be made, and the breeder must be aware of the relative importance of each trait for a given market. For example, low kernel row number may be acceptable in very early fresh-market hybrids, while a main season or processing hybrid with 12 or 14 kernel rows would be unacceptable, and should be eliminated in the first stage of evaluation. Likewise, an excellent processing hybrid that is extremely susceptible to common rust should be eliminated from consideration for use in the upper Midwest. Appropriate commercial checks are useful in guiding such decisions.

Most table quality traits can be rapidly and efficiently evaluated subjectively. Equipment is available to measure many traits, including sugar content, tenderness, and succulence.[24,179] These analyses are usually relatively expensive and time-consuming and, if they are performed, they are usually only done in later stages of testing. Yield and yield parameters are usually determined using objective measurements.

Timing of harvest is a critical consideration in hybrid evaluation since quality declines and yield (ear weight) increases with advancing maturity. Therefore, hybrids must be harvested at the same physiological stage to make accurate comparisons. This problem is further complicated if there is a lack of uniformity of silking within a hybrid. It may take up to 7 days for 90% of the plants in an individual hybrid to silk.[32] This range is certainly large enough to result in quality differences among ears of the same hybrid. Range of flowering time is affected by genotypes and environments.[32]

Methods for determining harvest date are available for sweet corn.[180–182] The first step in determining timing of harvest usually includes recording the date when a certain percentage of plants in each plot have exerted silk. Each plot is harvested after a predetermined number of days, heat units, or at a specific kernel moisture. Basing harvest dates on number of days is easiest. Because of variations in average daily temperature over the harvest season, however, it can result in harvest of hybrids at different stages of maturity. Harvesting of plots based on heat unit accumulation reduces this problem. Kernel moisture can be determined using either a vacuum oven or microwave oven[180,183] and is an accurate but time-consuming method.

Xenia, the direct effect of the pollen on the phenotype of the kernel, must be considered when evaluating sweet corn. It is obvious that hybrids of different endosperm types, such as *su1* and *sh2*, must be tested in different blocks in the field and these blocks should be isolated from one another, either by distance or by adequate borders consisting of the appropriate endosperm type. For an adequate evaluation of quality it is equally important to isolate *su1 se1* types from *su1* and partially modified hybrids from unmodified hybrids, e.g., *Su1su1 sh2sh2* from *Su1Su1 sh2sh2*. There is also

some evidence that xenia affects table quality and kernel appearance within endosperm types.[178] These effects are usually small and often ignored but can be reduced by growing multiple row plots and harvesting the center rows.

During each stage of testing the breeder must be sure that adequate seed supplies of hybrids are available for the next phase of testing. If seed is not available at least one season will be lost in testing that hybrid. Required seed amounts increase with each stage of testing as does the cost of producing the seed, emphasizing the importance of rigorous culling in early testing. Likewise, seed of new inbreds will have to be increased as their hybrids advance through the testing program, so that adequate supplies of the inbred are available to produce commercial quantities of hybrid seed as soon as the decision is made to commercialize the new hybrid.

C. Selecting for Special Traits

1. Eating Quality

Eating quality of the current generation of sweet corn hybrids is very good, and for new hybrids to be accepted they must be sweet, flavorful, and tender. If the breeding population consists of elite high quality germplasm, inbreds may be extracted and their quality may be evaluated during hybrid testing. However, if non-sweet germplasm is included in the parental material, an attempt should be made to select for tenderness and flavor. Non-sweet germplasm often has relatively thick pericarp and imparts off-flavors to its progeny. This is especially true of corns bred mainly for animal feed such as Corn Belt Dent. Taste testing can be effective in selecting for more tender pericarp[106] and eliminating undesirable flavors and textures. Corns selected for direct human consumption often have very thin pericarp and pleasant flavors.[157,168] Taste testing, however, can be valuable in identifying desirable segregates during inbreeding.

Taste tests are also important when incorporating endosperm mutants. In new backgrounds *sh2* often results in overpowering sweetness and taste testing is required to recover more subtle corn flavors. The presence of *se1* is difficult to detect visually but may be selected for by taste testing or biochemical assay. Off-tastes with fruity or perfumey flavors are sometimes detected in putative *se1* genotypes, and it is very important to eliminate such germplasm.

The methodology of organoleptic evaluation or taste testing has been studied intensively.[184] To be effective in a breeding program, however, large numbers of ears must be evaluated quickly, with repeatability, usually under field conditions. During inbreeding, some breeding programs remove the upper portion of self-pollinated ears 20 to 25 days after pollination. The removed portion is labeled with a number corresponding to the part remaining on the plant, uncooked ears are evaluated, and the remaining portion of the best ears will be harvested after seed maturity. Others simply bite the ear while it is still on the stalk and save seed from only the best ears. A minimal level of training is required so that evaluators can describe and score flavors and sensations in a similar manner. Taste evaluation during inbreeding is primarily to eliminate undesirable traits, off-flavors, and toughness, and these are relatively easy to identify. Taste testing can also be used to select for subtle corn flavors and textures, but this requires more training of testers.

There are devices and techniques for objective analysis of sugar levels (refractometers, sugar analyzers, chromatography) and pericarp thickness and tenderness (microscopy, tenderometers, shear-press).[24,179,185] However, each technique usually measures only one parameter and none can detect subtle flavors that result from combinations of many different components. Furthermore, few of these techniques are rapid and inexpensive enough to be used in large-scale inbred development programs, although they may be used in the evaluation of hybrids

2. Improved Pest Resistance

The genetics of resistance to insects and diseases in sweet corn is similar to those in field corn, and there are a number of comprehensive reviews of the subject.[141,186,187] Losses due to pests and

costs of controlling them, however, tend to be much higher in sweet corn than in field corn, for the following reasons:

1. Tolerance of consumers to damage on the ears, kernels, or husks is much lower in sweet corn.
2. Sweet corn is planted in environments with severe pest pressure, such as south Florida.
3. The planting season is spread over a longer period of time, increasing the potential for exposure and allowing pest populations to build. In the major processing states of Minnesota and Wisconsin many serious pests do not overwinter. However, corn planted late in the season, mid-June to July, is at a juvenile stage, and thus more vulnerable, when diseases such as common rust and maize dwarf mosaic virus move north. Field corn planted in April or May in the upper Midwest escapes these diseases.
4. Finally, as discussed previously, sweet corn has a high proportion of Northern Flint in its background, and Northern Flint as a race tends to have lower pest resistance compared with Corn Belt Dents.

There are many important diseases and insects that attack sweet corn, and it is unlikely that any one hybrid with acceptable table quality will have high levels of resistance to all of them. However, severity of specific diseases and insects varies according to region and time of season, and care should be given to developing hybrids that have acceptable levels of resistance to pests that they will encounter. Resistance to both pathogens and insects may be selected during inbreeding. Procedures for inoculation of most pathogens and insects that attack sweet corn have been developed and can be used during inbreeding.

a. Common rust

Common rust can cause serious yield and quality reductions in the upper Midwest processing region. Hybrids intended for planting in late May, June, or July require some level of resistance for acceptance by growers. Common rust also causes economic damage in the northeastern U.S. and in Florida. Two forms of resistance are available: *general* or *partial*, in which the size and number of pustules are reduced, and *specific*, in which a hypersensitive reaction restricts pustule development.[186] Hybrids with greater levels of partial resistance are available.[129,131] Partial resistance is under polygenic control, although the number of genes is probably relatively small.[130] Specific or single gene resistance conditioned by the *Rp1* locus is available in many hybrids and these hybrids dominate the late planted sweet corn crop. The *Rp1* locus is a complex locus consisting of duplicated sequences in which mismatching and unequal crossing over can occur during meiosis.[188–191] The high frequency of unequal crossing over has generated a large number of alleles,[191,192] and new allele combinations have been fixed and released.[193] The allele most frequently used today is the *Rp1-d*. However, in 1999 a race of common rust virulent on *Rp1-d* germplasm appeared in the Wisconsin and Minnesota. other alleles at *Rp1* (*Rp1-e, Rp1-f, Rp1-g, Rp1-i,* and *Rp1-k* as well as the *c* allele at the *Rp3* locus) have some utility.[194,195] Resistance due to alleles at *Rp1* and *Rp3* is usually dominant. A case of overdominance has been reported at another rust resistance locus, *Rp8*.[196]

Southern corn rust (*Puccinia polysora* Underw.) has been reported on sweet corn in the Midwest processing region but generally does not cause economic damage in the U.S.[186]

b. Northern corn leaf blight

Northern corn leaf blight can cause economic damage in sweet corn throughout the eastern half of the U.S., southern Europe, and Israel. Northern corn leaf blight is a serious problem during the main growing season in Florida, and hybrids intended for Florida production should have some resistance. Both partial and single gene forms of resistance exist for northern corn leaf blight.[197] However, races of the pathogen are able to overcome all commonly used single genes.[186,198,199] The polygenic form of resistance is effective regardless of races present.[186]

c. Stewart's wilt

Hybrids susceptible to Stewart's wilt should not be grown in regions where the disease is a regular problem. Stewart's wilt is spread by the corn flea beetle (*Chaetocnema pulicaria* Melsheimer)[186] and can be a problem anywhere the insect overwinters. Overwintering of flea beetles is severely limited when the sum of the mean temperature for December, January, and February is 32°C or below. If the sum of the mean temperature for those three months is above 37°C, Stewart's wilt can be serious problem.[186] Thus Stewart's wilt is usually not a problem in the major processing states of Wisconsin or Minnesota, but can be throughout much of the rest of the country. Resistance to Stewart's wilt is highly heritable and appears to be controlled by few genes.[186,200] Resistant hybrids are widely available.

d. Smuts

Common smut is widely distributed in the corn-growing regions of the U.S. and can cause serious economic damage on susceptible hybrids. It is not completely clear what environmental conditions or cultural practices favor the disease,[186] and therefore it is difficult to predict where or when outbreaks will occur. Resistance is polygenically controlled,[186] and susceptible germplasm is relatively easily eliminated in a breeding program.[67] A number of popular hybrids, however, are susceptible to common smut. Apparently such hybrids remain popular due to the variability of smut outbreaks.

Head smut has resulted in economic losses in Idaho, Washington, Oregon, and California. Its presence in seed production areas of Idaho is of particular concern because it can be seed-borne.[32] Certain fungicides applied as seed treatments or in-furrow effectively control the disease and may be required when the seed is shipped into certain countries.[67] Heritable differences in resistance to head smut exist among inbreds and hybrids.[32,186]

e. Maize dwarf mosaic virus

Maize dwarf mosaic virus (MDMV) is the most serious virus disease of sweet corn in the U.S., and production of sweet corn has been limited in areas where the virus overwinters. MDMV can also cause serious losses in late-planted corn in the northern tier of states. The virus is transmitted by aphids, but it does not overwinter in Minnesota and Wisconsin. Source of inoculum in the northern states is unclear.[186] The virus can be seed transmitted, and this could account for the presence of MDMV in northern production regions.[186] Depending upon the cross, resistance is controlled by up to five genes,[201] and hybrids with good resistance to MDMV are available.[202,203]

f. Stalk, ear, and kernel rots

Sweet corn is harvested around 20 DAP, which is well before the seed matures and the plant begins to senesce. Thus, stalk, ear, and kernel rots are usually not a problem in sweet corn production. However, stalk, ear, and kernel rots may cause serious economic losses in the production of sweet corn seed.[32] Similar to field corn, stalk rots may be due to both physiological stresses and pathogens such as *Fusarium*, *Diplodia*, *Gibberella*, and *Pythium*, and usually are due to a combination of the two. Genetic differences in stalk rot resistance exist, but mechanisms of resistance are complex and resistance is often correlated with other traits such as late-maturity.[186] Severity of stalk rots are affected by climatic conditions, cultural practices, presence of foliar pathogens,[204] and genetics of the host.

Kernel and ear rots caused by *Fusarium, Diplodia*, and *Gibberella* have always been a problem in sweet corn seed production and are an important reason why the seed industry is located in the desert climate of southwestern Idaho. *Fusarium* kernel rot is often associated with insect damage, and differences in susceptibility to *Fusarium* exist among sweet corn inbreds.[205] In supersweets, reduced germination and seedling dieback can result from *Fusarium* infection of the developing kernels.[205] Maternal effects are important in resistance to *Fusarium*, suggesting that the site of resistance may be the pericarp or the silk.[206] Under high humidity, ear and kernel rots are intensified. *Gibberella zeae* (*Fusarium graminearum*) ear rot has appeared on sweet corn grown for processing.[207,208]

g. Insects

European corn borer is the most serious insect pest in the major processing states of Minnesota and Wisconsin, while the corn earworm often causes more damage in other regions. Genetics of resistance to these pests has been the subject of intense investigation in field corn,[141] and breeding for resistance to the European corn borer and the corn earworm has been a major effort of some public sweet corn programs.[137,138,140] Genetic variation for resistance within adapted elite sweet corns, however, is low,[32] and inheritance is complex.[209] Natural sources of resistance outside sweet corn may have very poor table quality and other undesirable characters such as long tight husks or brown silks. Unlike most of the diseases mentioned above, there has been little success in breeding high quality hybrids with useful levels of resistance to these insect pests.

As mentioned previously, transformation technology has resulted in the incorporation of the *BT* gene from the bacteria *Bacillus thuringiensis* into commercial sweet corn hybrids.[165] The BT gene is effective in reducing damage by Lepidopteran insect larva,[165] but its use will depend on cost effectiveness and consumer acceptance.

D. Seed Production

1. Introduction

A fundamental objective of all sweet corn breeding programs must be that high quality seed of the new hybrid can be produced dependably and economically. Seed parents must produce acceptable yields of high quality seed with good emergence. They should also be easy to detassel. Seed parents should be adapted to mechanical harvesting and husking, with good lodging resistance and ear placement. Pollen parent must produce adequate amounts of pollen under varying environmental conditions; thus, resistance to heat and drought stress is desirable.

All commercial sweet corn is based on one or more defective endosperm mutants, and production of high quality seed is more difficult than for most other types of corn. The mutants are enzymatic lesions in the starch synthesis pathway, which change endosperm carbohydrate composition, and in nearly all cases result in reduced starch levels. Kernels are more angular and brittle than standard field corn. Furthermore, most sweet corn has been selected to have thinner pericarp, which may split or blister away from the endosperm under certain environmental conditions or in some genetic backgrounds. Thus, it is not surprising that germination, field emergence, and seedling vigor are often reduced relative to standard field corn. Reduced or uneven emergence results in reduced stands, lower yields, and variable ear size and maturity.

Reduced germination and seedling vigor are observed in many endosperm mutants, even in those that do not accumulate large amounts of sugar, such as *ae1*, *du1*, *floury2* (*fl2*), *opaque2 (o2)*, *shrunken1* (*sh1*), and *su1*.[210–213] Combinations of endosperm mutants usually result in even lower germination and seed vigor.[213,214] Mutants that do accumulate higher sugar levels at the expense of starch (e.g., *sh2*) have reduced germination relative to normal corn and other mutants, especially in cold soils.[215–217] Hybrids containing *se1* generally have reduced germination relative to *su1*.[218] Understanding the causes of poor field emergence in high-sugar types, and developing techniques to improve it, have been crucial in making high-sugar varieties commercially acceptable.

Genetic background strongly affects emergence, and background by endosperm mutant interactions is also important in determining emergence and seedling vigor.[213,214,219] Rowe and Garwood[213] studied germination and seedling vigor in three sweet corn inbreds, near-isogenic for 15 endosperm types. When averaged over endosperms, inbred Ia5125 had a germination percentage of 46%, while 81% of the Ia453 seeds germinated. In the same study, background by endosperm effects were observed (Table 6.7). Significant effects of genetic background on germination indicate improvement of germination is possible through selection. In a *sh2* population mass selected for improved emergence and seed weight, cold germination increased from 47% in cycle 0 (C0) to 64% in C10, while warm germination increased from 46% in C0 to 77% in C10.[98] Similar changes have been observed in a *su1* population selected for kernel phenotype.[97]

TABLE 6.7
Germination Percentage for Seven Endosperm Genotypes Evaluated in Backcross Conversions of Three Sweet Corn Inbreds

	Inbred		
Genotype	**S3-61**	**Ia5125**	**Ia453**
normal	94.0a[a]	92.9a	78.7ab
ae1	84.7abc	3.8d	66.2bc
du1	74.7abcd	59.8b	83.6ab
su1	70.0bcd	58.2b	90.2a
wx1	88.7abc	88.7a	86.2a
ae1 du1 wx1	38.9g	99.1cd	80.9ab
ae1 du1 su1 wx1	63.1def	62.2b	80.7ab

[a] Values followed by the same letter within a column are not significantly different.

Source: Rowe, D. E. and Garwood, D. L., in *Crop Sci.*, 18, 709, 1978.

Significant background by endosperm type interactions indicates that breeders cannot assume that backcrossing a new endosperm mutant into a line with good seed quality will result in a good germinating line.[67] Selection for good field emergence and seed type is advised during backcrossing.

2. Causes of Reduced Germination in *sh2* Corn

Supersweet varieties are widely grown throughout the U.S. and the world, but acceptance has been slow in some growing regions due to poor germination and inconsistent field emergence, especially in cold soils. These factors still limit the complete acceptance of supersweet corn varieties in the processing industry and the fresh market trade in the northeast.[219] Causes of reduced emergence are complex and strongly affected by both genetic and environmental conditions, both during seed production and at planting.[220,221] Seed weight of *sh2* is 33 to 50% that of *su1*[217,222] (Table 6.2) and seed weight is correlated to percent germination.[96,219] Reduction in seed weight is largely a function of greatly reduced starch levels relative to other types of corn.[90,223] Thus, starch levels are also related to germination and seed vigor both among endosperm types[223] and within *sh2*.[96]

On a percentage of dry weight basis, *sh2* seed has higher levels of sugars.[84,223] In an extensive study on effects of recurrent selection for improved germination in a *sh2* population, sugar levels were not directly related to germination.[96] However, due to the osmotic potential caused by higher sugar levels, *sh2* corn dries very slowly, and maturing seed is thus more susceptible to frost damage during maturation.[84] Slower drydown rates also may be responsible for the higher incidence of seed-borne *Fusarium moniliforme* Sheldon in *sh2* seed.[224] Infection by *F. moniliforme* and other pathogens can greatly reduce germination and seed vigor.[205] Styer and Cantliffe[223] demonstrated that *sh2* seed produced in the greenhouse had better germination and seedling vigor than field-produced seed. They attributed this to greater endosperm reserves and reduced pathogenic activity. While slower drydown rates of *sh2* seed may be responsible for increased infection of seed by *F. moniliforme*, Headrick et al.[225] have shown that increased infection is not directly related to higher sugar concentration.

Low carbohydrate concentration of *sh2* results in severe shrinking of the endosperm as it dries, which creates a number of structural problems for the seed. Shrinking endosperm pulls away from the pericarp, creating air pockets between endosperm and pericarp.[223,226] Air pockets increase the kernels' susceptibility to physical damage during handling and can result in cracking of the pericarp. The pericarp acts as a barrier to pathogens and water movement, both into and out of the kernel. Damaged pericarp greatly reduces germination in all endosperm types.[227]

Intact *sh2* kernels have a higher rate of imbibition than *su1* kernels, perhaps due to higher osmotic potential.[223] Higher imbibition rates, coupled with cracked pericarp, result in an in-rush of water, which may interfere with membrane reorganization.[228] Pericarp damage and disorganized membranes contribute to severe leakage of solutes from imbibing *sh2* kernels. Rate and amount of leachate, measured as electrolyte conductivity, are greater in *sh2* corn than in *su1*.[222,223,229] Seed leachate consists mainly of soluble carbohydrates (sucrose, glucose, and fructose), which are sources of metabolic energy for embryo growth during germination.[230] Leachate with high carbohydrate concentration may also encourage the growth of soil-borne pathogens and subsequent seed rots.

Seed leakage has been used in many species as an indicator of cold soil seed vigor and is one of the most effective seed vigor tests in both *su1* and *sh2* sweet corn.[222,223,229,231] After 10 cycles of selection for increased field emergence in a *sh2* population, seed leachate was highly correlated with improved emergence.[232] Seed leachate has been used successfully as a selection tool for increasing germination in *sh2* breeding programs. But care must be taken to ensure uniform maturity among the population of individuals to be evaluated as leachate levels are strongly affected by maturity. In a study by Borowski et al.,[233] seed leachate conductivity declined from 92 microamps at 72% seed moisture to 43 microamps at 23% seed moisture.

While *sh2* is only expressed in the endosperm, Styer and Cantliffe[234] presented evidence that *sh2* embryos may be dysfunctional. They found excised *sh2* embryos when grown in culture had poorer germination and growth than did embryos from *bt1*, *su1*, or normal seeds even when grown on the same media. This observation was not consistent with that of Wann,[217] who in a similar study found no significant differences among embryos when cultured. The two studies used the same hybrids and endosperm genotypes, and the differences in results are indicative of the importance of the seed production environment on seed quality.

Starch granule degradation in the subaluerone endosperm was significantly less in a *sh2* variety compared with non-isogenic *su1*, *su1 se1*, and normal varieties.[235] Thus, the availability of food for the germinating seed may be reduced. This observation has been made in only one *sh2* hybrid, but if this is generally true it could explain why even heavy seeded *sh2* genotypes often have relatively poor seed vigor. Both respiration rate and adenosine triphosphate (ATP) levels have been studied as factors in the poor seed vigor of *sh2* corn, and neither account for growth differences between *sh2* corn and other types.[216,217]

3. Improving Germination of *sh2* Seed

Selection for improved seed vigor in *sh2* corn has been a major effort of sweet corn breeders since *sh2* varieties were introduced. In addition to direct selection for germination under field and laboratory conditions, breeders have selected for increased seed weight, decreased electrolyte leakage, and decreased pericarp blistering. While no experimental evidence has been published, it seems that genetic gains in seed quality of commercial varieties have been made.

Perhaps more important in improvement of *sh2* seed quality are many refinements in seed production and seed treatments that have been implemented. The details of *su1* seed production were outlined by Huelson[24] in 1954 and changed little until the introduction of high-sugar varieties, both *se1* and *sh2*. However, slow drydown and susceptibility to mechanical damage of high-sugar sweet corn seed, especially *sh2* genotypes, resulted in many changes in seed production. Standard *su1* sweet corn seed is harvested around 30% seed moisture with a range of 10 to 50%,[67] whereas high-sugar types are harvested at 50 to 55%.[67] Kernels are very tender at higher moisture levels and are easily damaged during picking and husking. At 55% moisture, the husks and shank may be green, making snapping and husking more difficult than would be the case for lower moisture corn. Ear-pickers have been redesigned and remodeled to cause less damage. *Shrunken2* ears are not husked in the field as was common for *su1* corn. Instead, harvested ears are brought to a conditioning plant, where husks are removed by husking beds that are modified to handle high moisture, high-sugar corns.[67] The improved beds are less aggressive and shorter, moving husked

ears off the bed more quickly, resulting in less damage to kernels. Kernels are easily crushed or damaged by dropping, and high-moisture ears are significantly heavier than lower moisture *su1* ears. Accordingly, bin and hopper sizes, and drop distances have been reduced.

Artificial drying is a critical step in processing high-moisture seed. High-moisture seed is easily damaged by drying at higher temperatures (50°C) and lower temperature (35°C) drying results in better seed quality.[236] At harvest time, large volumes of seed must be dried rapidly, so the drying rate must be fast. Therefore, higher airflow rates through drying bins are critical. High moisture seed is more costly and time-consuming to dry,[67] thus it is important to fine tune temperatures and drying rates according to expected seed volumes.

After drying, *sh2* seed is brittle and irregularly shaped and the pericarp may be blistered. Due to irregular kernel shapes, the embryonic axis may protrude beyond the rest of the kernel, and such seed is very susceptible to mechanical damage. However, seed must shelled, milled, sorted, treated, and packaged. Seed must be transported between each process, increasing the likelihood of damage.[226] Each of these steps has been modified to reduce abrasion and drop distance, and increase seed quality.[67] The importance of these modifications can only be realized when one has examined dry *sh2* seed and recognized how defective for weight, shape, and pericarp integrity these seeds actually are.

Effects of soil-borne pathogens on germination of *sh2* seed and the need for chemical seed treatments were recognized early in the development of *sh2* as a crop.[237,238] Reliable and effective fungicides are absolutely required for high-sugar corns to be an economically viable crop. Many combinations of fungicides have been tested and some are effective. Certain fungicide combinations have been observed to increase germination under cold test performance three- to four-fold relative to the percent germination of the untreated check.[239] Three to five different fungicides plus an insecticide are commonly applied to commercially treated sweet corn seed.[67,240]

Nearly all sweet corn seed produced for the U.S., and for much of the world, has been produced in the Treasure Valley region of southwest Idaho.[67] Huelson[24] estimated that 85% of the seed crop was produced there in 1954. This area became important because of the desert climate with nearly optimal conditions for sweet corn seed production. Hot days, cool nights, and irrigation result in high yielding, productive plants. Low humidity results in a relatively disease-free environment and assists in the drying of the seed. Ear and kernel rots are reduced relative to seed produced under more humid conditions and seed quality is generally better.

VII. TYPES OF VARIETIES

Since the introduction of the first sweet corn hybrid, ‘Redgreen’ in 1924,[120] most sweet corn varieties have been single cross hybrids.[24,32] To increase seed quality or seed yields, some modified single crosses are sold, but the sister lines are usually very similar. A modified single cross is hybrid in which one of the parents usually the female is made by crossing two closely related sister lines. Single-cross hybrids were rapidly accepted by the sweet corn processing industry, due to the demand for uniformity. Uniform ear size and shape are particularly critical in producing high quality processed corn with good yield and recovery. The cutters that remove kernels from the cob are set for maximum recovery. If ear size is too small kernels will not be completely removed from the cob. On large cobbed ears, cutters go too deep, jamming the cutter and resulting in cob pieces mixing in with cut kernels, reducing quality. Uniform maturity is of equal or greater importance. Sweet corn quality is dependent on timing of harvest relative to heat units accumulated after pollination. If individual plants in a field silk over an extended period, some ears at harvest time will be overmature and of poor quality. If processors harvest before the earliest plants become overmature, many ears will be immature, yield per acre will decrease, and recovery and costs will increase due to harvest and transport of immature tonnage.

Some old open-pollinated varieties, such as Golden Bantam and Country Gentleman, are still sold, and hobbyists also maintain and exchange open-pollinated varieties. Brewbaker[157] is developing open-pollinated varieties for the tropics

VIII. MARKET REQUIREMENTS

Sweet corn is consumed in two ways, fresh or processed, and requirements for these uses differ. Requirements are not mutually exclusive and some hybrids are acceptable for both uses. Often hybrids are deficient in one or more traits that eliminate them from consideration for certain uses. Within both processing and fresh corn are market divisions that require specialized hybrids. Many of the requirements for hybrids have been discussed.

A. Fresh Market Hybrids

Corn for fresh consumption should have a higher number of usable ears per acre. Husks should be attractive, maintaining a glossy, dark green color with long flag leaves. The husks should be long enough to protect the ear tip. The appearance of husked ears is important, with the preferred type having good tipfill and at least 16 straight rows of narrow kernels. Kernel color varies according to growing region, as noted previously. Florida and most Asian markets require *sh2* endosperm. Fresh market growers in other regions of the U.S. use either *sh2* and *se1* hybrids. Fresh market corn may be harvested mechanically or by hand; hence, for a hybrid to be widely accepted the ears should be easy to remove from the plant.

If a hybrid is exceptional in some respect, compromises may be made on some of these other traits. Extremely early hybrids may have fewer kernel rows, lower yield, or poorer eating quality relative to main-season hybrids. Even in early hybrids, it is unwise to compromise on eating quality. In many instances, the earliest hybrids will be the consumers' first taste of local sweet corn for the season, and poor quality may reduce return sales.

B. Processing Hybrids

Corn for processing may be divided into whole kernel canning, cream-style canning, whole ear freezing, and whole kernel freezing. All processed corn must be adapted to machine harvest, husking, and cutting. Uniformity of maturity and ear type are extremely important.

Amount of cut corn produced per acre is the important yield parameter for whole kernel and cream-style corns. This is determined by tons of corn per acre and recovery per ton, which is determined by kernel depth. Kernel style is important in whole kernel corn with long, narrow kernels preferred. Glossy, bright yellow color is desired. Processed white corn should be snow white. Kernel tenderness is important in determining the quality of whole kernel corn. Flavor is also important, but sweetness may be enhanced by adding sugar during processing. Salt is usually added as well.[24] When *sh2* hybrids are canned, the amount of added sugar and salt is usually reduced relative to standard *su1* corn. Often no sugar is added to processed *sh2* corn.

Cream-style corn undergoes the greatest alteration during processing, with kernels scraped from the ear and starch added along with salt and sugar to the final product.[24] Thus many important traits in whole kernel corn such as kernel appearance, tenderness, and flavor are of lesser importance. *Shrunken2* was readily accepted for cream-style processing because the amount of added sugar can be reduced and kernel style was not important. Many of the first *sh2* processing hybrids did not have the refined kernel style desired for whole kernel processing.

In many ways requirements for whole ear freezing are similar to those for fresh market corn, with ears per acre being the major yield parameter, and ear appearance also being important. Ears are often cut into sections and cylindrical ears are preferred because they result in uniform sections. Husk appearance and tipfill are less important, because husks are removed and ear tips are usually cut off. Higher sugar types are useful for whole ear frozen corn because it is not possible to add sugar to enhance the flavor of the frozen product.

All processed corn is harvested, husked, cut, and moved through the factory by machines. Hybrids for processing must be highly uniform and adapted to meet the requirements of the processing operation. Ear placement should be high enough to allow efficient harvesting, and plants

should resist root lodging. Husks should be easy to remove and kernels should resist crushing. Ear shape must meet the needs of high speed automated kernel cutters, cylindrical with a slight taper at the tip being preferred. Curved ears will effectively eliminate the hybrid from consideration.

IX. POTENTIAL VALUE

It is difficult to determine, but it seems that per capita consumption of processed corn in the U.S. has increased slightly over the last 10 years. While consumption of canned corn decreased from 6.1 kg per person in 1974 to 4.7 kg in 1988, it has remained stable since 1988.[34,31] Frozen corn consumption increased by 1.2 kg per person over the same period[34] and seems to be continuing to increase, although directly comparable numbers are unavailable. Total production of processed corn, however, has increased by approximately 60% in the last 25 years.[34,31] The discrepancy is explained by the fact that substantial amounts of processed corn are exported to Asia and Europe. Japan, Hong Kong, Korea, and Taiwan are the major Asian importers with France, England, and Germany leading European importers.[33] After the U.S., leading processors include Canada, France, Japan, and Thailand.[33] Processing of corn will continue to increase in most of these countries, but given the climatic advantages and the technical expertise and infrastructure present in the U.S., export of processed corn should continue to grow, barring the erection of trade barriers.

Increased use of high-sugar hybrids for processing may also increase per capita consumption in the U.S. Sweetness of whole ear frozen corn and frozen whole kernel corn is enhanced by using high-sugar types. With *sh2*, higher quality canned whole kernel corn may be made without adding sugar or salt, thus creating a product more attractive to health-conscious consumers.

There seems to be a significant increase in both consumption and production of fresh market corn since the introduction of *sh2* hybrids.[33] Production data from USDA Agricultural Statistics Service[33] is sometimes confounded by changes in reporting methods or areas, so it is difficult to conclude the actual causes of these changes. However, as a result of the introduction of high-sugar corns the quality of corn available to the consumer is now greatly improved.

Interest in sweet corn for both fresh and processed consumption is increasing throughout the world, especially in the countries of the Pacific Rim and Western Europe. Thus the need for high quality seed will increase. Most of this seed will be produced in southwestern Idaho. Therefore, while the seed requirements for domestic needs may remain constant, demand for sweet corn seed for export should increase.

Sweet corn is a traditional summertime treat for Americans and a mainstay of the vegetable processing industry. It continues to increase in popularity around the world, and exports of seed and processed product are increasing. Development of high-sugar corns, especially *sh2* and *se1*, is in large part responsible for the increase in popularity. The initial discoveries and development of all the high-sugar types of corn were by geneticists and plant breeders at land grant universities.[1,63,66,74] New markets and improved hybrids were then developed by private seed companies. This combined effort has resulted in major new markets for sweet corn, and improved quality for consumers all over the world. It is fair to say, however, that development of high-sugar corns would not have occurred, or at least been greatly delayed, if it were not for the efforts of publicly supported plant breeders.

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7 Popcorn*

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I. INTRODUCTION

The origin of popcorn (*Zea mays* L.) is an important component of the ongoing discussions on the origin of all maize.[1–3] Popcorn is indigenous to the Americas and was unknown in Europe before the discovery of America. Its center of origin was more likely South and Central America than North America.[3] Erwin,[4] after critically reviewing historical references to popcorn, discussed the origin and history of popcorn and suggested that popcorn was a mutation of flint corn. In 1955, however, Brunson[3] suggested that archeological evidence and the fact that popping ability is a quantitative trait controlled by many genes made Erwin's hypothesis improbable. The discussion on the origin and relationship of popcorn to other types of corn and relationships within races of popcorn continues today. Goodman and Brown,[5] referring to 100 popcorn races, stated "The U.S. popcorns are far more complex than the U.S. flint, flour, and dent corns and will require much more study before their relationships are understood."

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The major trait that separates popcorn from all other types of corn is the formation of large flakes after the kernels explode in response to heating. This trait is referred to as *popping expansion*. As with other traits in corn, the distinction between the ability to either produce a flake or not to produce a flake in response to heating is not clearly defined. There are flint corns and some dent corns that will produce a flake when heated, but these flakes are very small relative to the flakes produced by the snack food known today as popcorn. Variation in this trait will be discussed in greater detail in a later section.

II. HISTORY

The development of popcorn into a commercialized industry in the U.S. is relatively new, occurring in the 1880s. Previous to 1880, there was no mention of popcorn in either early farm papers or early seed catalogs.[4] Botanists and farmers were probably familiar with popcorn, but it was mainly grown as a garden crop. By 1900, popcorn had become common in homes throughout the U.S. and, by 1912, the nation's commercial production had increased to 19,000 acres (7,700 hectares), mostly in Iowa.[6] Eldredge and Lyerly[7] provided data on popcorn acreages, yields per acre, price per hundred pounds, and gross return per acre for Iowa from 1925 through 1941. Until about 1925, 'White Rice,' 'Queens Golden,' and 'Japanese Hulless' were the varieties in commercial production, with White Rice being the most important[3] (see section on germplasm resources for additional early variety names). A few years later the variety 'South American,' along with 'Supergold' and its sister variety, 'Yellow Pearl,' became important in production and replaced Queens Golden.

The first variety used in the caramel popcorn industry was 'Spanish' popcorn, which is a flint corn that pops. Popping expansion was mediocre, but the large kernel and the tough texture of the flakes adapted this variety well to caramel corn processing. The Spanish variety was soon replaced in the caramel popcorn industry by better-expanding varieties of the South American type.

The first popcorn hybrid for commercial production was released in 1934 from the Minnesota Agricultural Experiment Station and was called Minhybrid 250. It was developed by H. K. Hayes and I. J. Johnson and was a single cross between two inbred lines, C1 and C6, developed from 'Michigan Pop,' a selection from Japanese Hulless.[8] Thus, the first popcorn hybrid was a sisterline single cross. It was adapted only to the northern edge of the U.S. Corn Belt and was not widely used by commercial growers. The first hybrids adapted to the central U.S. Corn Belt were released by the Indiana and Kansas Agricultural Experiment Stations in the early 1940s.[9] By the late 1940s, these hybrids had completely replaced the open-pollinated varieties for commercial production. In 1955, Brunson[3] listed the following hybrids that state agriculture experiment stations had released to the public for commercial production:

Minnesota
- Minhybrid 250 (white)
- Minhybrid 251
- Minhybrid 252

Kansas, in cooperation with the U.S. Department of Agriculture
- K4 (same as P32)

Indiana, in cooperation with the U.S. Department of Agriculture
- P20 P31 P38
- P22 P202 P32 (same as K4)

Iowa
- IOPOP5 (white)
- IOPOP6
- IOPOP7 (white)

Brunson[3] also stated that several private hybrids produced by commercial popcorn companies were available on the market. At this time, Minnesota, Kansas, and Purdue had released all the inbred lines in their hybrids to the public, but the Iowa Agricultural Experiment Station had not. In 1958, Brunson and Richardson[10] provided a table listing the pedigrees of public hybrids available through 1956.

An in-depth review and discussion of popcorn history, from popcorn's earliest beginnings to the popcorn we are familiar with today, and the U.S. popcorn industry are provided by Smith.[11]

III. THE POPCORN INDUSTRY

In 1998, the U.S. popcorn industry represented 3.8% of the total $43.5 billion snack-food industry in the U.S.[12] Since the early 1880s, growth of the industry has fluctuated. Popcorn consumption decreased in the 1940s after an increase in the number of home televisions. Until then, eating popcorn had become synonymous with going to the movie theater, so when movie theater attendance decreased because of home television viewing, so did popcorn consumption. At this time, the Popcorn Institute (a trade association of popcorn processors), realizing something had to be done, worked out an agreement with Coca-Cola* and Morton Salt companies to merge advertisements to convince consumers that popcorn was also good to eat while watching television at home. It was a good merger, because popcorn tastes good with salt on it, salt makes one thirsty, and Coca-Cola is a thirst quencher. These advertisements, coupled with the advertisements of popcorn companies, made the late 1940s and early 1950s the largest home-consumption growth period for the popcorn industry.[13] Going to the movies was a once-a-week pastime, but watching television at home occurred every night.

The U.S. popcorn industry has enjoyed nearly continued growth for the past 40 years, except for a few leveling-off periods (Figure 7.1). After a peak in 1993, the industry now appears to be in another leveling off period. One reason for this most recent leveling off period is the intense competition popcorn faces on grocery store shelves from other snack foods. To deal with this increasing competition, the popcorn industry has worked to institute a Popcorn Promotion Board that had its first meeting in 1999.[14] It seems that whenever people in the popcorn industry feel they have a mature market (steady demand over several years) or a leveling-off period something comes

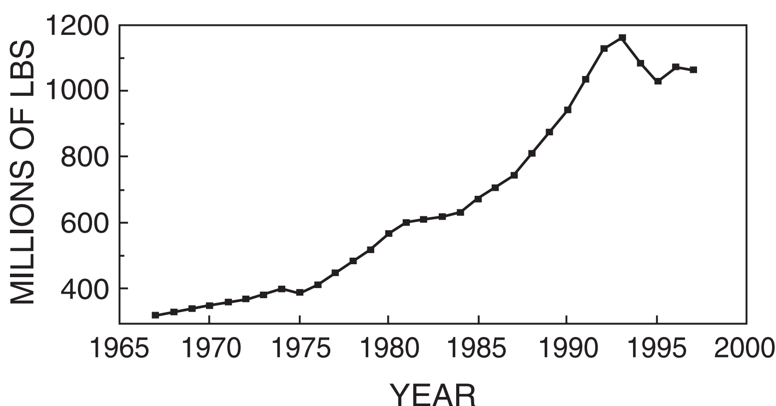


FIGURE 7.1 Sales of unpopped popcorn as reported by the Popcorn Institute, Chicago, IL, 1966 to 1997.

* Mention of a brand name, trademark, or proprietary product does not imply endorsement or recommendation of the product by the author or the Iowa Agric. Home Econ. Exp. Stn. and does not imply its approval to the exclusion of other products that may also be suitable.

along to provide new growth to the industry. Perhaps the future work of this board will be the springboard for new growth in the popcorn industry. In the 1980s, two factors were involved in providing growth in popcorn consumption. A new type of cooking, the microwave, was developed. Development of microwave technology for popping corn has resulted in microwave popcorn making up a major part of popcorn industry products.[12] At this time, too, Americans were starting to demand fast, easily prepared, healthy foods, a role that popcorn fits nicely.

Even though the sale of unpopped popcorn has shown nearly continuous growth on a year-to-year basis (Figure 7.1), popcorn production on a year-to-year basis shows considerable fluctuations (Figure 7.2). There are two principal causes for the drastic year-to-year fluctuations in production. One is the weather (growing conditions), and the other is that a majority of popcorn acres are grown under contract with processors. A year of bad growing conditions for popcorn is usually followed by an increase in acreage contracted by companies to increase their supplies. If that year is a good production year, there can be a large increase in production of popcorn. In general, though, the number of acres contracted by processors results in a consistent supply of popcorn for the industry. This concept of contracted acreage in popcorn production is important to the popcorn breeder developing hybrids. Because most contracts specify the hybrid to be grown, popcorn breeders have to market new hybrids to processors rather than to growers as dent corn breeders do.

The popcorn industry can be divided into two categories: groups involved with the production and processing of the raw popcorn (unpopped kernels) and groups involved in the use of the raw popcorn. Some larger popcorn companies are involved in both categories of the industry. Groups in the first category include seed companies, growers, pesticide producers, herbicide producers, fertilizer producers, popcorn processors, and packaging companies. Groups in the second category include brokers, truckers, popper manufacturers, microwave companies, prepop companies, coating companies (both salt- and sugar-based coatings), home consumer users, companies that package corn for home consumption, entertainment poppers (theaters, sports stadiums, fairs, etc.), the gourmet popcorn market, and novelty popcorn markets (colored popcorns, small kernel and small flake popcorns, and microwave popcorn on-the-ear). New uses for popcorn are constantly being developed and tested. Some are successful and others are not. It is important for the popcorn breeder to be aware of what is happening within all segments of the popcorn industry to provide the best direction for a breeding program. Each one of these segments places unique demands on the hybrids developed in a popcorn breeder's program, and thus on the breeding material used in the program.

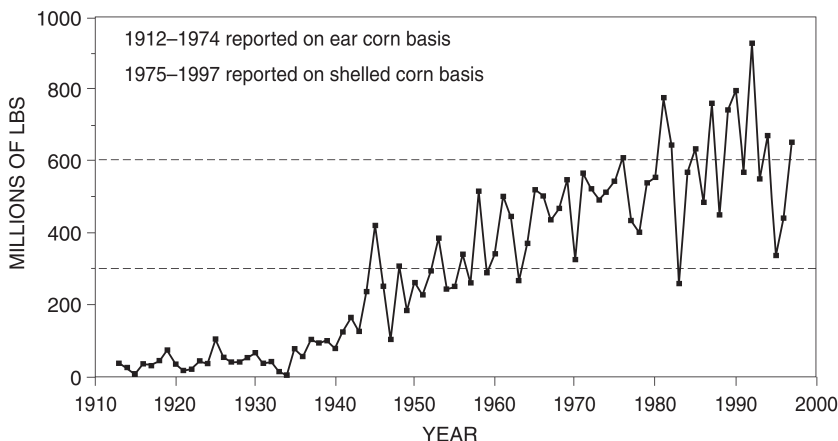


FIGURE 7.2 U.S. popcorn production 1912 to 1997 as reported by the USDA Crop Reporting Service to 1981 and the Popcorn Institute thereafter.

IV. GROWING POPCORN

A. Areas of Production

Commercial popcorn production has always been centered in the U.S. Corn Belt, but the major growing areas within this region have shifted over the years. From the 1880s through the mid 1940s, Iowa was the primary popcorn-producing state.[8,10] With the introduction of hybrids, production moved, first to Illinois and then to Indiana. The next major shift came in the 1970s, when production shifted to Nebraska.[15] The U.S. Department of Agriculture Crop Reporting Service dropped popcorn from their reports in 1981. Since 1981, the Popcorn Institute has published yearly information obtained from their members on U.S. popcorn acreage and production (Tables 7.1 and 7.2)

Indiana has ranked first for the past several years for total acreage and pounds of production. However, popcorn is grown over a large geographic area representing many different environmental production areas that presents challenges to the popcorn breeder in the development of hybrids adapted to these differing environments.

B. The Popcorn Plant

Popcorn is corn, so perhaps the best way to describe a generalized hybrid popcorn plant is to compare it with a generalized hybrid dent corn plant. Figure 7.3 depicts some of the major differences. The popcorn tassel is larger than a dent corn tassel, with the tips of the branches hanging down, giving the popcorn tassel a "weeping-willow" appearance. The popcorn tassel, because of its increased size, produces much more pollen than the dent corn tassel. Even though the drawing shows the popcorn plant to be shorter than the dent corn plant, there are popcorn hybrids that are as tall as, or taller than, some dent corn hybrids. The top ear placement in popcorn tends to be higher than the ear in dent corn. Popcorn is prolific, with most hybrids producing more than one completely developed ear. The ear shoots and ears of popcorn are smaller than those of dent corn. The popcorn stalk tends to be thinner and less sturdy than the dent corn stalk. Because of this thinner, weaker stalk, popcorn tends to be more prone to stalk

TABLE 7.1
The 1997 Popcorn Institute Popcorn Production Study of the Popcorn Acreage Planted and Harvested in the U.S.

	Acreage planted[a]			Acreage harvested[a]		
State	**1995**	**1996**	**1997**	**1995**	**1996**	**1997**
Illinois	19.8	27.7	36.1	19.8	27.0	35.7
Indiana	63.5	58.6	81.9	63.5	56.6	81.2
Iowa	3.4	4.0	5.7	3.2	4.0	5.7
Kansas	5.2	4.0	5.4	5.2	4.0	5.4
Kentucky	4.8	7.3	8.3	4.8	7.1	8.2
Michigan	0.1	1.1	0.6	0.1	1.1	0.6
Missouri	0.0	4.3	14.1	0.0	4.3	14.1
Nebraska	8.4	14.7	25.1	7.8	14.6	24.4
Ohio	11.1	8.5	13.1	11.1	8.1	13.0
Other states[b]	6.3	8.3	55.3	5.7	7.7	51.8
Total	122.6	138.5	245.6	121.2	134.5	240.1

[a] Thousands of acres (convert to thousands of hectares by dividing by 2.471).

[b] Colorado, Maryland, Minnesota, New Jersey, North Carolina, Oklahoma, Oregon, Pennsylvania, South Dakota, Tennessee, Texas, Wisconsin

TABLE 7.2
The 1997 Popcorn Institute Popcorn Production Study of the Total Pounds of Production of Popcorn and the Pounds of Yellow and White Types Produced in the U.S.

	Yellow[a]			White[a]			Total[a]		
State	**1995**	**1996**	**1997**	**1995**	**1996**	**1997**	**1995**	**1996**	**1997**
Illinois	62,189	93,876	103,505	160	160	301	62,349	94,036	103,806
Indiana	151,949	169,187	229,503	2,760	1,536	1,611	154,709	170,723	231,114
Iowa	5,726	8,988	12,229	2,194	1,724	2,828	7,920	10,713	15,057
Kansas	17,662	16,194	24,512	0	0	0	17,662	16,194	24,512
Kentucky	14,747	20,879	23,291	0	0	75	14,747	20,879	23,367
Michigan	324	3,168	1,797	0	0	0	324	3,168	1,797
Missouri	0	12,371	41,199	0	77	0	0	12,449	41,199
Nebraska	21,644	46,905	86,703	3,802	8,762	10,514	25,446	55,667	97,216
Ohio	29,965	25,101	47,227	679	0	179	30,644	25,101	47,406
Other States[b]	20,883	31,537	64,642	930	774	766	21,813	32,311	65,407
Total	325,089	428,206	634,608	10,525	13,033	16,274	335,614	441,241	650,881

[a] Thousands of pounds (convert to thousands of kilograms by dividing by 2.2).
[b] Colorado, Maryland, Minnesota, New Jersey, North Carolina, Oklahoma, Oregon, Pennsylvania, South Dakota, Tennessee, Texas, Wisconsin.



FIGURE 7.3 Diagrams of dent corn and popcorn hybrid plants to illustrate morphological differences.

lodging. Popcorn also is more susceptible than dent corn to many of the pests of corn. Figure 7.3 suggests adventitious roots on the popcorn plant, but this tends to be the exception rather than the rule. Only under the correct growing conditions will popcorn produce adventitious brace

roots and, even then, they are not as pronounced as in dent corn. Leaf shape and size vary as much in popcorn as they do in dent corn, but the popcorn leaf generally is narrower, with a more upright orientation.

Popcorn yields measured on a weight-per-land-area basis are less than dent corn yields. Bushel comparisons can be made, but should be avoided because the standard test weight of popcorn is 65 lb/bu (826 kg/m^3), whereas the test weight of U.S. Grade No. 1 field corn is 56 lb/bu (712 kg/m^3). Also, popcorn yields are reported adjusted to moisture levels from 13.0 to 15.0%, but dent corn yields generally are reported adjusted to 15.5% moisture. In the industry, popcorn yields are discussed in pounds per acre (kilograms per hectare), compared with bushels per acre (metric tons per hectare) in dent corn.

A major difference between popcorn and dent corn is the shape and size of the kernels. Popcorn kernels are smaller and harder than dent corn and contain a much greater hard endosperm to soft endosperm ratio (Figure 7.4).[10] Actual pictures of cross-sectional cuts of five different types of corn kernels with various ratios of hard to soft endosperm are provided by Watson.[16] Popcorn also has the thickest pericarp (the outer covering of a corn kernel) of all types of corn.[17] There are two shapes of popcorn kernels, the rice type and the pearl type. Rice types tend to be long and slender with a sharp point at the top; pearl types are more round with a smooth top. A majority of the commercial hybrids are pearl types.

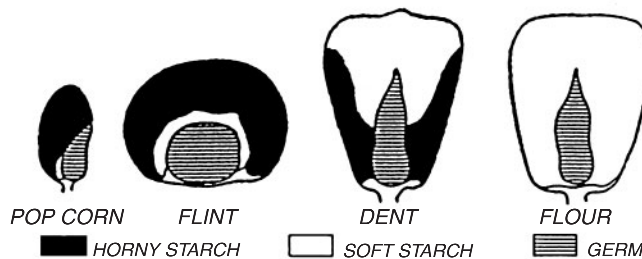


FIGURE 7.4 Location and relative proportions of hard and soft starch in the four main classes of starchy corn. (From Brunson, A. M. and Richardson, D. L., Popcorn, USDA Farmer's Bulletin No. 1679, 1958.)

The phenology and growth of popcorn in comparison with dent corn were studied in detail by Stevens et al.[18] In one study, they compared the growth of popcorn hybrid IOPOP12 with the growth of dent corn hybrid B73xMo17. They reported that staminate and pistilate spikelet development in B73xMo17 and in IOPOP12 followed a similar sequence, but relative to leaf stages, IOPOP12 tended to lag one or two emerged-leaf stages behind B73xMo17. In general, when popcorn and dent corn hybrids are planted on the same date, in the same field, and at the same depth, popcorn hybrids emerge later than dent corn hybrids.

C. Cultural Practices

Limited experimental data are available regarding the cultural practices of growing popcorn. A general overview of cultural practices for the commercial crop was presented by Ziegler et al.[15] Generally, the cultural practices used by dent corn breeders for growing dent corn[19] can be, with minor modifications, used by popcorn breeders for growing popcorn. Some of the minor changes result from plant-type differences discussed in the previous section. Popcorn tends to be slower emerging, so any practice that can speed emergence is desirable (i.e., warmer seed bed with good kernel-to-soil contact and adequate moisture). Because of popcorn's slower germination and seedling growth, timely planting is critical. For maximum popping expansion, popcorn must reach harvest maturity before the first hard frost. Soil fertility values recommended for dent corn in a localized growing area are generally satisfactory for growing popcorn. Any dent corn fertilizer

program being adapted to popcorn growing should be adjusted to reflect popcorn's relatively poorer standing ability. This is especially true in the more advanced yield trials of a breeding program. Greater rates of nitrogen application may compound lodging problems. Experienced commercial popcorn growers apply somewhat less fertilizer than recommended for dent corn, reasoning that, because yields are less, less fertilizer is needed.[15] At comparable plant densities, this is probably a safe practice. At greater popcorn plant densities, this may not provide adequate nutrients. Because of its lesser yield and smaller plant size, popcorn usually is planted at a greater plant density than dent corn, ranging from a 0 to 25% increase beyond the recommended dent corn planting rates. Stevens[20] evaluated the effect of increased plant densities on the popcorn hybrid IOPOP12. Yields increased quadratically from 3,646 lb/acre (4,088 kg/ha) at 13,600 plants/acre (34,000 plants/ha) to 4,787 lb/acre (5,467 kg/ha) at 30,000 plants/acre (74,000 plants/ha) and then declined as the density increased to 38,000 plants/acre (94,000 plants/ha). Plant densities in a breeding nursery will depend on the type of material being worked with and the data being collected.[19]

Many of the same pests that have to be dealt with when growing dent corn also have to be dealt with when growing popcorn. A description and discussion of popcorn pests are presented in the *Popcorn Pest Management Manual for the Midwest*.[21]

Espada and Bedin[22] evaluated the effect of duration of weed competition on the growth and yield of popcorn hybrids in the Philippines. In their study, plant heights, percentage of plants with ears, and yield significantly decreased with extended duration of weed competition from 0 to 13 weeks after planting. Mechanical weed-control methods,[23,24] such as rotary hoeing and cultivating, are the same for popcorn as for dent corn. Some chemical weed controls (herbicides) can damage popcorn at high rates and improper application times. The best approach when using chemical weed-control methods is to follow label directions carefully. When using chemicals, the popcorn breeder needs to be especially careful because of the poorer plant vigor of inbred material. Genetic engineering techniques have developed dent corn germplasm that is resistant to some herbicides. This technology can be licensed and used by popcorn breeders to incorporate herbicide resistances into popcorn. These new herbicide resistances, while providing more control over weed pests, make weed control issues more complex for the popcorn breeder. Today a decision needs to be made to either incorporate or not incorporate these genetically modified herbicide resistance traits into working popcorn germplasm.

Insect pests for popcorn are the same as those for dent corn, as discussed by Benson and Pearce[24] and Dicke and Guthrie.[25] The most destructive insect pest of popcorn is the second-generation (feeding on the leaf sheath, collar, and stalk) European corn borer, [*Ostrinia nubilalis* (Hübner)] (ECB). Jarvis et al.[26] showed that even a very light infestation of ECB can result in considerable yield losses. A detailed discussion of this pest in popcorn will be presented in a later section.

As with weeds and insect pests, the diseases common to dent corn are also common to popcorn[15] and are discussed in detail by Smith and White.[27] Also, as with dent corn, the states that grow popcorn each have their unique set of diseases to consider. Stalk and root rots, however, are the most destructive diseases of popcorn and occur over the entire popcorn growing area. This disease complex generally is caused by several fungal and/or bacterial pathogens rather than by a single causal agent.[15] A study in Nebraska[28] showed that, in 1981 and 1982, *Fusarium moniliforme* Sheld was the principal organism in the stalk-rot disease complex in popcorn.

The popcorn breeder needs to decide whether to control some or all pests in the nursery or use natural infestation as natural selection pressure on the material. If, however, local pest problems are severe enough to drastically reduce yields and seed set on hand-pollinated material, some control measures must be used.

With Nebraska being one of the major popcorn-producing states, irrigation of popcorn is an important topic. Popcorn requires 18 to 24 in. (46 to 61 cm) of water during the growing season. Assuming other growth factors are not limiting, about 125 to 150 lb (57 to 68 kg) of grain are produced for each acre in (2.5 cm) of water applied.[15] Shortage of water at any time during the growth period can result in reduced yields, but water deficiency during tasseling and silking is the

most detrimental. This is especially true in a breeding nursery because moisture stress can delay silk emergence enough that some crosses and self pollinations can be missed. In dry production areas, additional moisture after pollen shed can make the difference between average yields and excellent yields. Under dry growing conditions, irrigation should continue until the black-layer of the kernel has formed.[29] Generally, if irrigation is the accepted practice for dent corn production in a growing area, it also will be necessary for popcorn production.

Before-harvest cultural practices for growing popcorn are basically the same as for growing dent corn. Starting with harvest, however, differences become more pronounced. If popcorn is to be combine-harvested, most contracts for commercial popcorn state that it must be left standing in the field until the grain reaches a moisture percentage of at least 18%, and sometimes 16%. This is the timeframe, therefore, that the breeder should try to harvest advanced evaluation trials. Harvesting within this timeframe is necessary to prevent mechanical damage to the popcorn kernel. Any break, scratch, or scuff in the pericarp of a popcorn kernel reduces the popping volume.[30,31]

If commercial popcorn is ear-picked with a mechanical picker, it can be harvested at moistures up to 25%. Popcorn can be hand harvested anytime after it reaches physiological maturity, assuming adequate drying facilities are available. A popcorn breeder must decide how to harvest popping samples from advanced hybrid yield trials (i.e., hand or machine harvest). The final decision will depend on an individual breeding program's objectives. The subject of damage from combine harvesting popcorn was reported on by Lien and Haugh[32] and Lien et al.[33] They showed no loss in expansion when the combine was set optimally. Use of combine-shelled samples from advanced experimental tests could be a means for breeders to select material that shells more easily, assuming, along with optimum combine operation, that the material in the experiment is of similar maturity.

Popcorn kernels must be dried slowly to prevent stress cracks from developing, but fast enough to prevent mold development.[34,35] This usually involves large amounts of air and no additional heat. Popcorn should not be dried below 11% moisture. If dried below 11% moisture, the popcorn can be rehydrated, but its potential popping expansion will not be realized unless rehydration occurs very slowly.[36,37] Commercially, the optimum procedure to produce the greatest popping expansion is to dry the popcorn down to 14% moisture on the ear, shell it, clean it, and moisture proof seal it to stabilize the moisture content.

New-crop popcorn needs to be conditioned before it can be popped. Conditioning refers to drying the popcorn to optimum moisture content (13.5 to 14.0%)[6,35] and holding it there for at least 4 to 6 weeks, or longer if possible. A popcorn breeder has the options of conditioning the harvested corn on the ear or as shelled corn. Conditioning on the ear can give slightly greater popping expansions, but the choice between these two generally depends on availability of space in a conditioning chamber, a room with controlled temperature and relative humidity. A relative humidity of 70% and a temperature of 70°F (21°C) will condition popcorn to 12.5 to 13.5% moisture, with different genetic material coming to equilibrium at slightly different moisture contents. Four to 6 weeks are needed to ensure that every kernel in a sample is near the same equilibrium moisture content to provide the most accurate popping expansion data (i.e., all the kernels pop in about a minute).

A breeder conditioning popcorn on the ear can harvest at any moisture content between physiological maturity and 11% moisture to obtain maximum popping expansion, assuming adequate air flow through the conditioning chamber to prevent mold growth at the greater moisture percentages. Usually, ears are picked between 15 to 25% moisture and moved directly to a conditioning room, where they hang in mesh bags for a minimum of 4 weeks before shelling and sealing in moisture-proof containers. Ashman,[37] in a study relating popcorn drying regimes to popping expansion, suggested that overdrying, followed by rewetting, might be a better approach for the breeder than drying to the optimum moisture content of 13.5 to 14% and holding it at that moisture content. In his study, the popping expansion curve of rehydrated samples of two hybrids compared with the expansion curve of dried samples of the hybrids showed less-pronounced peaks over moisture contents (Figure 7.5 and 7.6). Thus, the exact moisture content

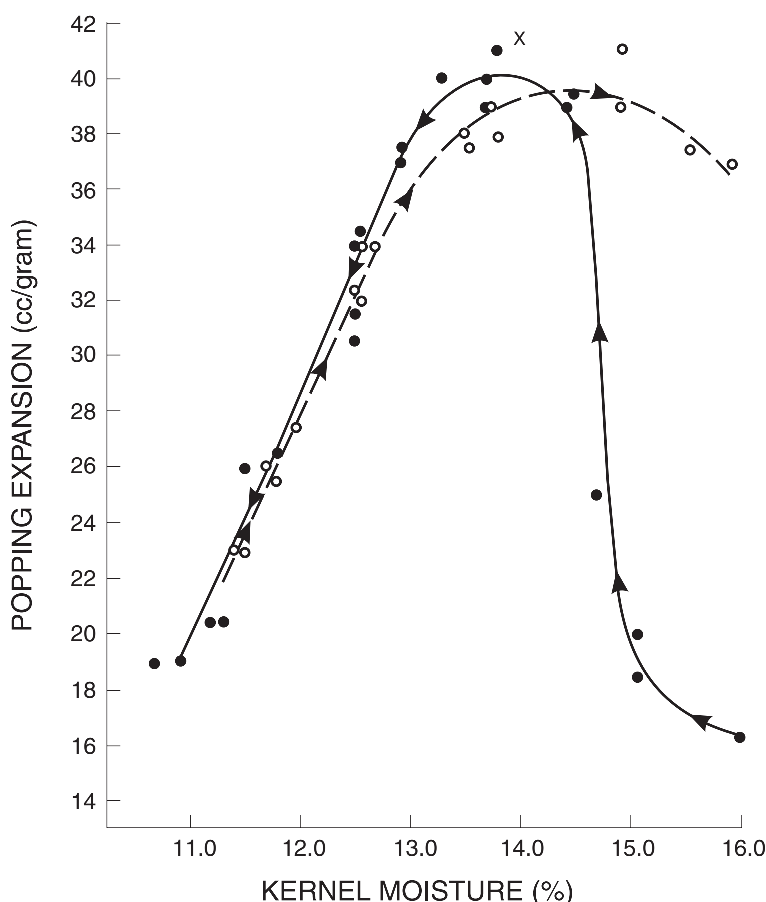


FIGURE 7.5 Relationship between popping expansion volume and percentage kernel moisture for popcorn hybrid 43449 (released as P405). Solid-circle data points were samples of shelled popcorn taken during drying, and open-circle data points were samples taken from overdried shelled popcorn that had been rewetted; solid- and broken-line smooth curves were fitted to the respective data points. The "X" data point is from an ear-dried sample.

for rehydrated samples may not be as critical when comparing popping expansions of a large number of samples and this may help remove a source of variability in expansion data. In a following study, Ashman[38] showed a hysterisis effect (equilibrium moisture content of grain for a certain set of temperatures and humidity conditions is different depending on whether the process is desorptive or adsorptive), but that the overdrying and reconditioning had no detrimental effect on popping expansion when the rehydration was done over a period of four weeks at 70% relative humidity and 70°F (21°C).

An option for the breeder with a smaller conditioning facility is to condition the popcorn as shelled corn. This involves hand harvesting the breeding nursery between physiological maturity and 11% moisture, with the normal practice being harvesting between 15 and 25% moisture; hanging the ears in mesh bags in an open area; shelling the ears between 13 and 16% moisture; and placing the shelled samples in a conditioning room maintained at 70% relative humidity and 70°F (21°C). The moisture content of the hanging ears before shelling needs to be monitored closely so that it does not decrease below 11%. Fall weather conditions of a specific geographic area play a major role. In a dry fall, additional humidity may need to be added to the hanging area to prevent

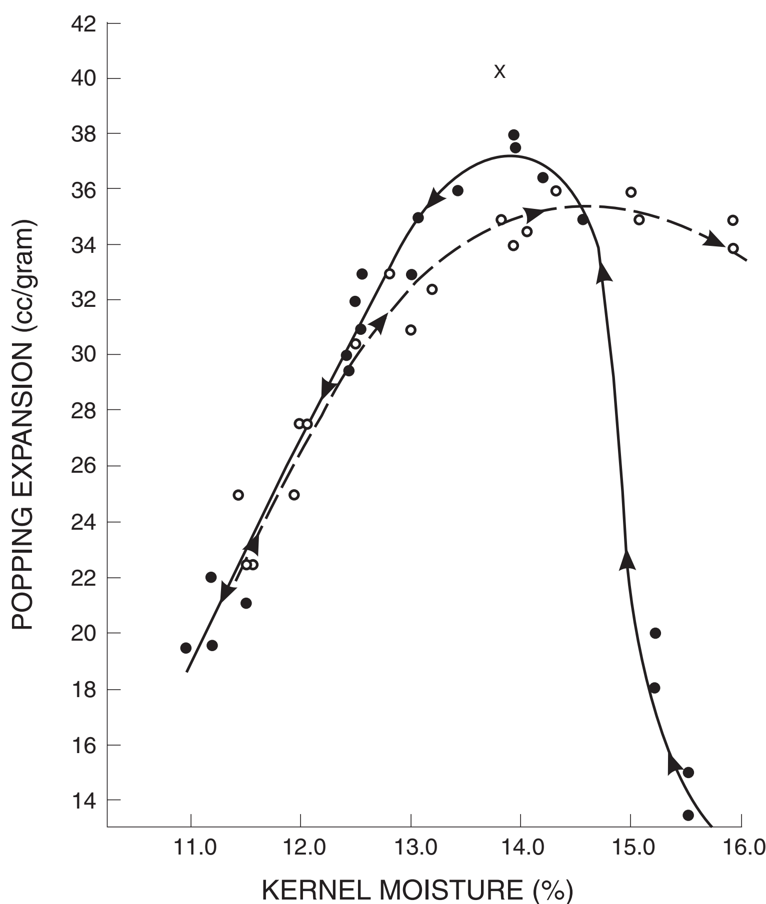


FIGURE 7.6 Relationship between popping expansion volume and percentage kernel moisture for popcorn hybrid 73286. Solid-circle data points were samples of shelled popcorn taken during drying, and open-circle data points were samples taken from overdried shelled popcorn that had been rewetted; solid- and broken-line smooth curves were fitted to the respective data points. The "X" data point is from an ear-dried sample.

excessive drying. Adsorptive vs. desorptive conditioning practices also must be considered.[38] With this method, material should be sorted by maturity so that the earliest material can be shelled first to prevent or reduce overdrying. Within an individual experiment, material should always be treated the same to minimize sources of variation.

Storage of commercial popcorn production is a topic critical to the delivery of a quality product, but it is not part of the focus of this chapter. In a popcorn breeding program, after the popcorn grain has been conditioned and popping expansion data collected, the popcorn breeder needs to store the grain to be used for seed and discard the open-pollinated grain used to determine popping expansion from performance trials. If popcorn is left in the conditioning chamber too long, great numbers of stored-grain insects can develop. For the grain that is to be used for seed, the popcorn breeder is no longer concerned with maintaining popping expansion moisture contents of 13.5 to 14%, but is concerned with maintaining good germination. Suggested long-term cold storage conditions to maintain germination for corn are 45 to 50% relative humidity at 50°F (10°C).[19] Cooler temperatures and lower humidities are desirable for long-term storage, but are hard to achieve because of the cost of more elaborate environmental control equipment.

D. Seed Production

Seed production in popcorn is similar to seed production in dent corn.[39,40] There are three areas, though, where slight differences occur. The differences in these three areas are a result of the poorer agronomic performance of popcorn inbreds as compared with dent corn inbreds; the utilization of the gametophytic incompatibility gene, $Ga1^s$, in popcorn; and the seed size and shape differences between popcorn and dent corn.

The poorer agronomic performance of popcorn inbreds relative to dent corn inbreds suggests that more care is needed in popcorn seed production fields. Slower growing, less vigorous popcorn inbred lines do not compete as well against weeds as dent inbred lines do, so more emphasis must be placed on weed control. Because some popcorn inbreds are more susceptible to herbicide damage, greater attention must be placed on choice of herbicide. Yields of popcorn inbred lines are less than those of dent corn inbreds, one of the reasons some commercial popcorn hybrids are still three-way crosses. The smaller ear size of popcorn inbred lines necessitates additional harvest machinery adjustments. If seed fields are irrigated, the poorer root growth of popcorn inbred lines can require additional applications of irrigation water. The tendency of some popcorn inbred lines to tiller causes additional detasseling work. Also, the greater susceptibility of popcorn inbred lines to lodging can affect harvesting procedures in a popcorn seed production field.

In popcorn, utilization of the gametophytic incompatibility gene, $Ga1^s$, (dent sterility discussed in a later section) means that isolation distances for popcorn seed production fields can be adjusted accordingly. Popcorn seed production fields where the seed parent is dent sterile, $Ga1^s$-$Ga1^s$, can be planted adjacent to dent corn fields with no concern for contamination. More concern, however, must be exercised for isolation distances from other types of corn, such as sweet corn, that could carry the $Ga1^s$ gene. When in doubt whether the potential contaminating field carries the $Ga1^s$ gene, isolation distances similar to those recommended for dent corn should be used: typically, 410 to 660 ft (125 to 201 m) from similar corns and 660 ft when the contaminating corn has a different kernel color or endosperm type.[39]

Kernel size and shape differences between popcorn inbred lines and dent inbred lines result in differences in cleaning, sizing, grading, and bagging procedures. The smaller seed size of popcorn necessitates the use of different screen sizes in the cleaning process. Popcorn seed is sized into small, medium, and large sizes and listed as kernels lb^{-1} (0.45 kg), with 3,900 to 4,000 being small, 3,000 to 3,900 medium, and 2,500 to 2,900 large. Most growers prefer a range of 2,800 to 3,800 kernels lb^{-1}, using 3,500 kernels lb^{-1} to estimate the number of bags needed to plant their acreage. Because of these different sizes, most popcorn seed is bagged in 50-lb (22.5 kg) bags for sale, compared with the 80,000-kernel bags used in dent corn. Because of the shape of the popcorn kernel, there is no length grading of the seed and no flat grade. All popcorn seed is graded as rounds.

Forms of cytoplasmic or genetic male-sterility systems used in popcorn seed production are limited, but the economic potential of using male-sterility methods in popcorn seed production fields for pollen control is similar to dent corn seed production. Wych[39] describes these methods for dent corn, and Thomas and Johnson[41] and Thomas and Eldredge[42] discuss the use of cytoplasmic male sterility in popcorn. A listing of references pertaining to cytoplasmic sterility (specifically Texas male sterility) is provided by Levings and Williams.[43] Because of the potential economic value of male sterility in corn seed production, new methods of developing male sterility are continually being proposed and evaluated.

To assist seed producers, popcorn breeders strive to improve yields, pest resistances, competitive growth traits, herbicide tolerances, and to increase ear and seed size of inbred lines. Also of benefit to seed producers, popcorn breeders can verify that inbred lines and breeding populations released are homozygous for $Ga1^s$, the dent sterility gene, and keep informed of research on male sterility so that when a workable system is identified, it can be incorporated into breeding material. To be successful, popcorn breeders must develop inbred parental lines acceptable to seed producers that

require no delayed planting of either parent and produce hybrids that perform well in all categories of maturity and seed size and under all popping systems.

V. BREEDING POPCORN

A. Procedures and Techniques

The procedures and techniques for breeding popcorn are similar to those for breeding dent corn.[19] There are a few areas, though, where some minor adjustments are needed. These adjustments are necessary because of the poorer agronomic traits of popcorn germplasm compared with dent corn germplasm. Three main reasons for the poorer agronomic traits of popcorn include the following: limited effort (person years) has been focused on improvement of popcorn compared with dent corn; agronomic traits of popcorn are not the most important traits emphasized in selection; and the base germplasm available for popcorn breeding programs does not perform agronomically as well as the base dent corn germplasm. These poorer agronomic traits in popcorn can alter cultural practices used by popcorn breeders in their nurseries compared with those used by dent corn breeders.

Probably the most notable technique difference in breeding popcorn is that usually the ear shoot leaf needs to be removed before an ear shoot bag can be placed on the shoot. Ear shoots of popcorn are small and often are only 1 in. (2.5 cm) above the leaf collar when silks appear. Under some conditions, silks may appear before the ear shoot appears. Another trait that differs somewhat from dent corn is that most popcorn is prolific, and the breeder needs to be sure to shootbag the upper ear shoot. This can be difficult at times because, in some popcorn germplasm, second ear development can be slightly ahead of top ear development. For this reason, shoot bagging in a popcorn nursery usually takes longer than in a dent corn nursery, because the breeder has to pull the next-higher leaf collar away from the stalk to see if there is an ear shoot at a higher node. Another noticeable difference in a popcorn nursery is the amount of pollen produced. Popcorn is an excellent producer of pollen, with the tassels having many more branches than dent corn. Because of this, pollen shed from an individual popcorn tassel can cover a longer period of time than pollen shed from a dent corn tassel. Another trait that can differ is that popcorn ear shoots are often found higher on the plants than dent corn ear shoots.

B. Germplasm Sources

Sources of information about the very early history of popcorn are sketchy, as discussed by Smith,[11] but it is likely that the first popcorn breeders were Indian tribes of Central and South America. These tribes' practice of parching corn to make it more palatable might have been the technique that identified the first popcorn. Popcorn seems to be a flint corn modified by selection to maximize popping expansion.[44] Once it was identified, these early breeders must have maintained the seed, for when Columbus discovered America, popcorn was known and used by Indians of the Americas.[8,10,11]

These early popcorn populations are likely the predecessors of the germplasm sources breeders use today. In the late 1800s, when popcorn became a commercial crop, the more prominent varieties were Japanese Hulless, White Rice, Queen's Golden, Spanish, 'Superb,' and 'Tom Thumb.' In the late 1930s, when popcorn hybrids started to become available, these populations were the major germplasm sources used to develop the first inbred lines. The first popcorn hybrid released in 1934 was the result of crossing two inbred lines from the same Japanese Hulless variety. Descriptions of these early varieties and some of the early hybrids are included in several publications.[3–8,10,45,46] Since the early years of inbred development, some open-pollinated varieties have proven to be of more use than others as sources of inbred lines: 'Supergold,' 'South America,' 'Amber Pearl,' 'Ohio Yellow,' White Rice, and Japanese Hulless.[47] Other open-pollinated varieties that have not contrib-

uted to present-day commercial hybrids include Queen's Golden, Spanish, 'Strawberry,' 'Argentine,' Tom Thumb, 'Black Beauty,' 'Ladyfinger,' and 'Red.' Some of these varieties, however, have contributed germplasm to novelty popcorn markets.

Presently, popcorn breeders utilize many different sources of germplasm, depending on the goals of the breeding programs. Because popcorn is a member of *Zea mays*, any germplasm within *Zea mays* can be used. In a chapter about maize germplasm, Hallauer and Miranda[48] provide a detailed discussion of the members and races of *Zea mays*. Extensive collections of the different cultivars, types, and races of all corn are available at the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), El Batan, Mexico; the National Seed Storage Laboratory, Fort Collins, CO; and the North Central Regional Plant Introduction Center, Ames, IA.[19] Of course, the more nonrelated the germplasm is to popcorn, the longer it will take to derive useful materials. Popcorn breeders in the early 1940s realized that dent corn germplasm could contribute to popcorn germplasm, especially for some agronomic traits.[49] As expected, popping expansion and quality were reduced, but breeders found that these traits could be recovered after backcrossing to the popcorn parent.[49–51]

Present-day popcorn breeders have several options for material to use as sources of inbred lines within popcorn germplasm. The original popcorn varieties or popcorn germplasm from other countries can be used, but these have the general problems of nonimproved traits or of not being adapted to the growing area. These populations may, however, contain unique genes that can make it worthwhile to spend the additional time needed to move the genes into usable material. Popcorn breeding programs at Purdue University, Iowa State University, and the University of Nebraska have released several breeding synthetics: Purdue—HPXD-1, HPXD-2 and HPSGDS; Iowa—BSP1C1, BSPW1C1, BSP2C1, BHPXD-1C2, BSPM1C1; BSP3C1, BSPM2C1, BSP1C4, BSP4APC0, BSP5C0, BSP6CBC0, and BSP2C4; Nebraska—YPILFWS(1), SGIILFWS(2), NPSGS1(3), NPSG1GaS1(2), NPYPIS1(3), and NPSGTTS1(3). The HPXD-1 and HPXD-2 synthetics from Purdue represent popcorn germplasm with dent corn in their background and HPSGDS is a source of dent sterility in Supergold background. BSP1C1, BSPW1C1, and BSP2C1 from Iowa represent synthetics of commercial hybrids. The BHPXD-1C2 is Iowa's version of Purdue's HPXD-1 after two cycles of recurrent selection for improved popping expansion. BSPM1C1 is a specialized synthetic developed to provide a source for inbred lines with the mushroom popping trait. BSP3C1 is a source of germplasm with dent corn in its background. BSPM2C1 is source of germplasm producing a high frequency of mushroom flakes with dent corn in its background. BSP1C4 is cycle 4 of recurrent selection in BSP1C1. BSP4APC0 is an improved breeding synthetic related to Amber Pearl types. BSP5C0 is an improved breeding synthetic related to South American types. BSP6CBC0 is an improved breeding synthetic related to South American types with a high level of tolerance to the second generation of the European corn borer. And BSP2C4 is cycle 4 of recurrent selection in BSP2C1. Two additional Iowa State University populations are scheduled for release in 2000 that will be an improved South American breeding synthetic and an improved Supergold breeding synthetic. The Nebraska population YPILFWS(1) is an improved version of the open-pollinated popcorn variety, Yellow Pearl, and the SGIILFWS(2) population is an improved version of the open-pollinated popcorn variety, Supergold. YPILFWS(1) and SGIILFWS(2) have undergone one and two cycles of selection for Goss's wilt and blight disease [*Clavibacter michiganese* spp. *nebraskense* (Shuster, Hoff, Mandell, and Lazar) emend. Vidaver and Mandell (syn. = *Corynebacterium nebraskense*)] resistance, respectively. NPSGS1(3) is the maintained version of a 1940s Supergold variety, NPSG1GaS1(2) is a dent sterile version of Supergold, NPYPIS1(3) is a Yellow Pearl type, and NPSGTTS1(3) is derived from Supergold crossed to Tom Thumb.

Licensing of germplasm from another company can be another means for a popcorn breeder to expand the genetic base of a popcorn breeding program. Advances in genetic engineering represent the development of another source of germplasm for popcorn breeders. Popcorn breeders with sufficient resources can license genetic engineering developments and incorporate them into popcorn.

C. Breeding Methods

All the breeding methods applicable to dent corn improvement can be used in a popcorn improvement breeding program. For dent corn, these methods have been discussed in detail.[40,48,52,53] Progress in improvement of popcorn agronomic traits has been slower than in dent corn because, in addition to agronomic traits such as yield, stalk strength, disease resistance, and insect resistance, popcorn breeders must also consider quality traits such as popping expansion, freedom from hulls, freedom from objectionable flavors, and texture and tenderness of the flakes. These additional quality traits in popcorn breeding are under genetic control, with popping expansion, the most important trait, dependent upon the presence of four or five major genes.[9,54] The quality traits will be discussed in detail in later sections.

As with dent corn,[53] mass selection was used to improve popcorn. The Supergold variety, for example, resulted from an early mass-selection experiment to improve popping expansion.[3,8] When popcorn hybrids began to be used, breeding methods similar to those used for dent corn inbred-line development also were used in popcorn improvement programs to develop inbred lines. The choice of breeding method, or methods, used is dependent on the goals of each individual breeding program, the germplasm being used, and the resources available. Another factor that might affect the choice of breeding method for popcorn improvement is that some popcorn hybrids today are still three-way crosses, compared with dent corn, in which most present-day hybrids are single crosses. The main reason for the development of three-way crosses in popcorn is the poorer agronomic performance (especially seed yields) of popcorn inbred lines relative to dent inbred lines. As popcorn inbred line performance has improved, there has been an increase in popcorn single crosses grown commercially.

VI. TRAITS

A. Types of Popcorn

Different types of popcorn as raw products move through commercial channels, with each type filling a specific need of the end user.

The two main kernel-color types of commercial popcorn are yellow and white, with yellow being the most important commercially. In a breeding program, depending on the germplasm used, all gradations of kernel color from bright white to dusty white and from lemon-yellow to bright orange can occur. If popcorn kernels are to be packaged and sold in clear containers, the bright whites and bright orange types have more eye appeal. Within the species *Zea mays* many different kernel colors can be found. All of these colors can either be found in popcorn or crossed into popcorn. Two of these kernel colors, black (deep purple) and red, are presently being marketed on a small scale as popping corn. Hybrid vigor (heterosis) can be expected when crosses are made between the different color types because of the diversity of the original source populations.

Within the yellow kernel popcorns, the raw product moves through commercial channels in three kernel sizes: small, medium, and large. There are no industry standards for kernel size determinations, but a commonly used measure is based on the number of kernels in 10 grams: 52 to 67 = large, 68 to 75 = medium, and 76 to 105 = small.[15] Home consumers generally prefer the small kernel types because small kernels usually produce more tender flakes with few hulls. Vendors tend to prefer the larger kernel types because large kernels produce larger flakes that have good eye appeal and are tougher, to reduce breakage from handling, even though they may have more hulls. The medium kernel size hybrids can be used by both end users.

There are two distinct shapes of popcorn kernels, described as rice or pearl types. Rice types have long kernels with a sharp point at the top and are historically associated commercially with white popcorns. Pearl types are round with no sharp point at the top and are historically associated commercially with yellow popcorns. Because of intercrossing of the two types in breeding programs,

both shapes can be found in yellow and white germplasm, but the commercial trend has moved away from rice-type hybrids and more toward pearl-type hybrids.

The popped product of a popcorn kernel is called a flake. There are two distinct flake types that are commercially important: butterfly and mushroom. Butterfly hybrids produce flakes that are irregular in shape with many "wings" coming out in all directions. This type of flake tends to be associated with tenderness and freedom from hulls. However, they will not withstand much handling because the flakes easily break into smaller pieces. Because popped popcorn is commercially sold by volume, this is not desirable to vendors who sell the popped product, but some vendors will sacrifice product shrinkage for tenderness and freedom from hulls. Vendors in the popcorn coating industry — whether it be candy, salt, or other flavor coatings — prefer mushroom type flakes. Mushroom hybrids produce flakes that are round with only a few wings to break off during handling. Although these two flake types are affected by growing environment and popping environment, they are controlled genetically. There are popcorn hybrids that produce 100% butterfly flakes, but no commercially available hybrid, at present, produces 100% mushroom flakes; however, in 1998, a couple of experimental hybrids developed at Iowa State University and grown at several locations were scored at 100% mushroom flakes.

There are currently three options for popping popcorn: heated air, heated oil, or microwave. In a comparison of oil popping and microwave popping, Dofing et al.[55] have shown a genotype-by-popping-method interaction. There are different hybrids that perform better under different popping regimes. This has created a marketplace demand for hybrids that perform well under microwave popping conditions, others for oil popping, and still others for heated-air popping conditions. Microwave popping has become a major part of the popcorn industry. For the popcorn breeder this means that emphasis must be placed on selection of material that performs well under microwave popping conditions.

There also are many different novelty types of popcorn, such as Strawberry, Ladyfinger, Argentine, Tom Thumb, and Indian popcorn (uniquely colored popcorn). These types presently are not commercially important, but some, like black popcorn and red popcorn, are being commercially marketed on a small scale as popping corn. Even though the kernels may be many different colors, the flakes are white or yellow, with the hull keeping its original color in the popped sample.

A popcorn breeder must decide what percentage of the overall program, if any, is to be spent on the improvement of each of the different types of popcorn. Because of this demand for all the different types of popcorn, a popcorn breeder must consider and evaluate the following traits:

Kernel and flake color (visual identification of color)
Kernel size [kernels per 10 grams: 52 to 67 = large, 68 to 75 = medium, 76 to 105 = small, >105 = very small (novelty)]
Kernel shape (visual identification of shape)
Flake type (visually rated as percentage of mushroom flakes occurring in the popped sample with 0% mushroom equal to 100% butterfly)
Microwave vs. traditional popping expansions (determined by choice of popping method used)
Novelty popcorns (visual identification of unique popcorn types).

B. Quality Traits

Matz[56] discusses the basis of quality in popcorn. It is difficult, however, to develop one definition of excellent quality popcorn because each of the different end-user groups involved in the popcorn industry define excellent-quality popcorn on the basis of their own needs. A popcorn consumer's definition of excellent-quality popcorn is a product that has good appearance, flavor (taste), and texture, and freedom from hulls and unpopped kernels. A popcorn vendor's definition of good-quality popcorn would include these same traits, but also would include popping expansion. The

candy or salt-based coating companies prefer popcorn that has a mushroom flake, whereas companies that sell to home consumers prefer popcorn with a butterfly flake. In general, though, the most important quality trait that will be evaluated by a popcorn breeder is popping expansion.

Large popping expansion is the major trait that separates popcorn from other types of corn. It is controlled by four to five major genes (a quantitative trait), with several minor genes contributing to other characteristics, such as shape and tenderness of the flakes and hull breakage and dispersal.[9] Even though other types of corn, such as some flints, will pop, none has the expansion volume of popcorn. Heritability estimates for popping expansion have ranged from 0.62 to 0.96.[57–59] Weaver and Thompson[57] and Robbins[58] provide reviews of the literature on the nature of inheritance of popping expansion.

What makes popcorn pop? This has been asked many times and has been discussed in detail by Hoseney et al.,[30] da Silva et al.,[31] and Matz.[56] There are two events that occur when popcorn pops — it explodes and produces a flake. The two factors controlling the explosion part of the phenomenon are moisture in the kernel and an intact pericarp. Upon heating, the moisture inside the kernel expands to a point where enough pressure is built up to burst the pericarp. Anything that changes either of these two characteristics from their optimum state reduces popping volume. The build-up of internal pressure provides the driving force for the production of a flake. The expanded flake is derived from the translucent portion of the endosperm. During popping, starch granules do not explode, but upon release of internal steam pressure are gelatinized and expanded by heat, dried, and left in a three-dimensional network. The soft endosperm, or soft starch granules, undergo very little change during popping except to spread farther apart. Even though the soft endosperm does not expand, it may play some role in expansion, because even after many years of improvement in popping expansion, present-day popcorn still contains some soft endosperm. The production of large flakes is associated with the ratio of hard (translucent) to soft (opaque) endosperm. Popcorn produces a flake and other corns do not because the popcorn kernel has the right ratio of soft to hard endosperm, with a pericarp strong enough to confine the internal steam pressure until it is great enough to provide the optimum driving force to expand the translucent endosperm starch granules. Thus, the popping phenomenon is a function of the physical structure of the entire kernel and the microscopic structure of the endosperm.[55]

The industry standard measurement for determining popping expansion is cubic centimeters of popped flakes produced by a gram of unpopped kernels. The accepted industry standard instrument for measuring this trait is the Metric Weight Volume Tester (MWVT) produced by Cretors and Co., Chicago, IL. Matz[56] discusses previous standard testers and makes some comparisons among them. The MWVT determines expansion under the oil popping regime and requires a 250 g sample of popcorn. Present-day acceptable popping expansion volumes commercially are 40 cc/g or greater. With the majority of the popcorn used today being popped in the microwave, the Technical Committee of the Popcorn Institute developed a standard protocol for popping corn in the microwave.[60]

These methods are useable for advanced generation material in a breeding program in which a larger sample of popcorn is available. For earlier generation materials, however, in which much smaller samples are available (for example single ear samples), there are no standard methods for measuring popping expansion. Robbins[58] provides a literature review on the use of single-ear expansion data in breeding programs. Various methods have been used to gather expansion data on small samples, with some being more successful than others. These methods include popping corn in an earlier version of the standard popcorn tester developed by Cretors and Co., which required only 150 g per sample;[56] adding a charge of a known hybrid to the sample to be tested to bring the total sample size up to the amount needed for either of the standard poppers; changing the heat and voltage settings on the standard poppers to pop small samples without scorching them;[61] making adjustments to commercial home poppers to withstand continuous popping; dry-popping kernels in a bowl in the microwave; and popping small samples in a pan over a stove or hot plate. Ashman[62] discusses several of these methods and indicates advantages and disadvantages of some of them.

Another concern of the breeder when popping small samples is in deciding the most appropriate way to measure the popped flake volume. As discussed by Ashman,[62] this is not a minor concern and can have a large effect on the data collected. Contrary to his results, however, the Iowa State University program has had fairly good success popping a 30-g sample in an Amana Radarange (RCS720B) commercial microwave and measuring expansion in a 3-in. (7.62 cm) 2000-ml graduated cylinder.

A breeder wanting to gather expansion data on small samples (single ears) needs to experiment with various methods to decide which method works best. If none of the methods mentioned previously is acceptable, the breeder may have to reconsider gathering expansion data on small samples or try some new approach to popping small samples. The major requirement is that the results correlate well with standard popping methods. Hybrids do perform differently under different popping regimes.[55,62,63] The method used to pop small samples also must be consistent within each set of material from which selections are to be made. Popping expansion data from various sample sizes and volumetric cylinders do not respond well to mathematical conversions. This became apparent when the switch was made from the Official Weight Volume Tester (OWVT), which measures the expansion of a 150 g sample in cubic inches per pound, to the MWVT, which measures the expansion of a 250 g sample in cubic centimeters per gram. Because of the way flakes filled the calibrated cylinders and the different quantity of flakes, it was not possible to apply a mathematically calculated conversion to arrive at the same magnitudes of popping expansion values for popcorn samples evaluated in the different poppers. It was necessary to develop a conversion chart, based on a comparison of popping expansion data from the same material popped in both poppers, so popping expansions between poppers could be compared.[64]

Popping expansion is the only quality trait of popcorn that can readily be measured but, as discussed, methods used to measure it can vary. This variation in the measurement of popping expansion, the hybrid by popping method interaction, and the different needs of different end users make developing a good quality popcorn hybrid a challenge for the popcorn breeder.

Other quality traits in addition to popping expansion provide information to the breeder about quality of popcorn — freedom from hulls (hull dispersion) and the shape, color, texture, and flavor of the popped flakes. The hull, the part of popcorn that gets stuck in the consumer's teeth, is the pericarp of the kernel.[56] Among all corns, popcorn has the thickest pericarp;[17] the thickness is necessary to contain the pressure required to produce a flake. Mohamed et al.,[65] after evaluating several physical characteristics of popcorn kernels, reported that for microwave popping and conventional popping, pericarp thickness had the greatest effect on popping expansion. In selecting for freedom from hulls, the popcorn breeder is not as much selecting for a thinner pericarp as selecting for a pericarp that shatters into small pieces during the explosion, as opposed to a pericarp that has just a few fissures and leaves big pieces of pericarp in the popped sample. In crosses between dent corn and popcorn, the pericarp is tough enough to contain the pressure, but the pericarp splits in half (one fissure) on popping, leaving big half hulls in the popped sample, which results in poor-quality popcorn. In breeding programs, hull dispersion can be visually rated on every popped sample, with the actual rating scale used dependent on program objectives and materials evaluated. After several years of microwave popping 5,000 to 10,000 individual ears per year in the Iowa State University popcorn breeding program, it has become obvious that if selections are based on popping expansion alone without including information on hull dispersion, the end result will be high expansion, hully material. If selection indices are used, hull dispersion ratings should be included.

The shape of the popped flakes — whether they are butterfly or mushroom type — is, as mentioned earlier, important to the end user. Even though the formation of mushroom flakes is affected by environmental conditions, moisture content, and popping regimes, it also is subject to genetic control. Recurrent selection for the mushrooming trait in two popcorn synthetics was initiated at Iowa State University. After only one cycle of S_1 recurrent selection in BSPM1C1,[66] improvement was made in the population's ability to produce mushroom flakes. This trait is visually

rated by assigning a percentage value of mushroom flakes to each popped sample. In 1996, in the Iowa State University popcorn breeding program, the first lines to pop 100% mushroom flakes were identified.

Flake color, texture, and taste are important quality traits but are not routinely rated in most breeding programs. Flake color is either yellow or white, with white-kernel popcorn producing white flakes and yellow-kernel popcorn producing yellow flakes. Most of the colored novelty popcorns produce white flakes, but the colored pericarp and/or aleurone give the popped sample an off-white color. For data collection on color, generally, anything different from normal is noted. Texture (tenderness) is correlated with freedom from hulls (good hull dispersion) and a greater percentage of butterfly flakes.[56,67] A consumer's definition of acceptable texture is crispy flakes that melt in your mouth as opposed to chewy flakes that tend to stick to teeth. Because texture is positively correlated with popping expansion, freedom from hulls, and formation of butterfly flakes, very little additional data are routinely collected on this trait except on the most advanced elite experimental hybrids.

Taste (flavor) is difficult to define because every person's likes and dislikes are different. Also, aromatic, as well as palatability (texture), factors are involved. Routine data generally are not collected for this trait except to cull out the bad-tasting or bad-smelling material. Only in advanced elite experimental hybrids is taste evaluated, and then usually not by professional tasters. Slightly different from taste, but associated with it, is the aroma of popping popcorn. Aroma is complex, with pyrazines, furans, pyrroles, carbonyls, and substituted phenols all contributing to the flavor and aroma of popping popcorn.[68–72] There are aromatic rice varieties that smell like popcorn when cooked.[69] After a study of the compounds involved in this popcorn-like aroma, Paule and Powers[70] suggested that a breeding program for aromatic rice should include selection for greater concentrations of 2-acetyl-1-pyrroline and less hexanol. Pinson[73] discusses the inheritance of the popcorn-like aroma in rice and reports that Ahn et al.,[74] using RFLP technology, had mapped a single aromatic recessive gene in the rice variety Della to chromosome 8. A method of analysis for 2-acetyl-1-pyrroline has been developed by ARS scientists at the Western Research Center in Albany, CA.[75] Historically, popcorn selections were made on the basis of traits from samples popped in oil. Any selections made for flavor (taste) were based on the interaction of the popcorn flavor with the oil flavor, with the oil flavor masking much of the popcorn flavor. For many years, popcorn hybrids may have been selected for less flavor because this lack of flavor would have interacted less with the different flavors of the oils used then. More recently, some consumers, for health reasons, need to eat popcorn without oil added (dry popped). Under this popping regime, some present-day hybrids have limited flavor. Perhaps by utilizing the 2-acetyl-1-pyrroline analysis method, popcorn breeders could enhance the popping aroma (flavor) of new popcorn hybrids. Routinely, though, popcorn hybrids are selected for the absence of bad flavors or bad taste.

Little information is available on the nutritional qualities of popcorn. Recently, though, with snack consumer's increasing awareness of nutritional and health issues, an increased interest in nutritional quality traits of popcorn is occurring. As the popcorn industry strives to increase popcorn's share of space on grocery shelves, in the near future, popcorn breeders may also want to evaluate nutritional traits in their programs.

Many factors play a role in the final outcome of these quality traits of popcorn. Anything that affects the growth and development of the popcorn plant and the harvesting, drying, shelling, cleaning, conditioning, packaging, storing, and popping of the kernels can have an effect on the quality of the popped flakes. In the popcorn industry it is an accepted fact that some years (environments) produce better-quality popcorn than other years. But, starting with the harvesting of the crop, the procedures and techniques used by the breeder can affect the quality of the popped flakes, and thus can play a role in the success of the selection schemes used. In outlining procedures and techniques for a breeding program, a popcorn breeder must resolve whether to strive for conditions that provide for the optimum popped quality of his material or to develop procedures and techniques that reflect commercial practice. The answer will be determined by the goals of the

individual breeding program and the type of germplasm being evaluated in the breeding program. In many instances, different germplasm will be handled differently within the same program.

C. Relationships of Traits Affecting Quality

Many of the nongenetic factors affecting popping expansion have already been discussed. Most of the sensory quality traits of popcorn are positively correlated with expansion (popped flake volume).[56,58,76]

In wide crosses of popcorn to other types of corn, initial gains in popping expansion can be made by selecting for popcorn-type kernels, suggesting that, in this type of material, kernel shape, size, and amount of hard endosperm are associated with high expansion. Ashman[77] evaluated dent × pop material and reported a significant (p = 0.05) negative correlation between kernel size and popping expansion of F_3 progenies. Experience has shown that, if selection pressure is too intense before intermating or backcrossing, the recovered material will be very similar to the original popcorn material.

Haugh et al.[78] reviewed previous literature and discussed research on the physical properties of the popcorn kernel and related these physical properties to popping expansion within 100% popcorn germplasm. Traits included moisture content, test weight, kernel size (kernel weight), kernel shape (kernel specific gravity, kernel volume, kernel sphericity), and kernel location on the ear. Different hybrids had different optimum moisture contents for maximum expansion, and the range of optimum moisture contents was different for individual hybrids. The average moisture range that gave the greatest expansion of the 12 hybrids evaluated was 13.5 to 14.8%. In their study, hybrids with greater test weight had greater expansion volume. Another positive trend reported was that hybrids having greater expansion tended to have greater specific gravities. The other traits evaluated showed no specific trends with greater expansion. Ziegler[79] reported no correlation between kernel hardness and popping expansion in HPXD-1, and a nonsignificant (p = 0.05) correlation (r = 0.30) in BSP1C1. HPXD-1, described by Ashman,[80] is a popcorn with dent corn in its background and BSP1C1, described by Ziegler,[81] is 100% popcorn germplasm. In Ziegler's study, data were collected on kernels from 100 S_0 ears from each population. Hardness data were collected by using an instrument similar to a drill press with a strain gauge attached to it. Data collected were the number of kilograms necessary to crush a kernel, with 10 kernels sampled from each ear. Pordesimo et al.[82] reviewed literature on the relationship of physical properties of the popcorn kernel with popping expansion. In their study, they evaluated the relationships of sphericity, kernel size, specific gravity, and elastic deformation of popcorn kernels with the performance of popcorn when popped in microwave bags. Varieties with sphericity values (as defined in their study) greater than 0.70 had a greater expansion value. Within an individual variety, large kernels resulted in lower unpopped-kernel ratios. Also, within an individual variety, a greater specific gravity resulted in greater expansion volumes and flake size. Elastic deformation, as measured in their study, had no correlation with microwave bag popping quality. Mohamed et al.[65] reported that expansion volume correlated positively with average pericarp thickness, sphericity, and Stenvert hardness.

Song et al.[83] reported that kernel size within a hybrid had a significant effect on popping volume, and that the middle-size fractions had the highest popping expansion and the lowest number of unpopped kernels. For a popcorn breeder though, interest in kernel size is driven by an increasing demand for large-kernel hybrids. Because of the way popping expansion data are generated, bulk volume of flakes from a set weight of unpopped kernels, selection based only on popping expansion tends to result in smaller kernel size. Since the collection of kernels per 10 g data is routine in most breeding programs, the mathematical conversion of popping volume data and kernels per 10 grams data into a popping volume for a single kernel is straightforward. The Iowa State University popcorn breeding program's use of this trait, individual kernel popping volume, has resulted in the selection of larger kerneled genotypes that would have been discarded if selection had been based

solely on popping volume. Selection based on this trait can also identify material for the relatively new marketing outlet requiring popcorn hybrids that produce large individual flakes.

Ashman[77,84] evaluated the relationship of total protein percentage, zein protein percentage, and oil percentage of popcorn kernels to popping expansion. The protein data came from a 2-year study on the effect of applying additional nitrogen to the soil in which popcorn was grown.[84] As the rate of nitrogen applied per unit area of the field increased, percentages of total protein and zein protein in the harvested popcorn kernels increased significantly, but expansion stayed the same. A follow-up study[77] on segregating material with a greater range of popping expansions also showed no significant correlation between total protein percentage or zein protein percentage with popping expansion. Nonsignificant correlations of popping expansion with oil percentage were 0.14 for 10 commercial hybrids and –0.13 when 25 S_0 ears from a dent × popcorn population were evaluated.[85] Thomas-Compton et al.[86] evaluated total protein percentage and lysine percentage of popcorn and derivatives of crosses between CIMMYT's quality protein maize hard-endosperm populations and popcorn. It seems the opportunity exists for improving popcorn nutritional quality while maintaining acceptable popping expansions.

Experience gained in working with and growing popcorn hybrids has shown a tendency toward an association of greater expansion with poorer agronomic traits. But, agronomic data collected from 1987 to 1990 in the Iowa State University breeding program from an evaluation of entries including approximately 100 S_1 lines from each of several synthetics, check hybrids, and a few lines in various stages of selfing had the following significant ($p = 0.05$) correlations with popping expansion: 1987 (500 entries) — yield = –0.2, maturity = 0.2, max (a measure of stalk strength) = 0.2, and P1 (a different measure of stalk strength) = 0.2; 1988 (148 entries) — maturity = –0.3 and max = 0.2; 1989 (341 entries) — maturity = –0.2; and 1990 (509 entries) — yield = 0.4, maturity = –0.1, plant height = 0.2, dropped ears in stalk rot inoculated material = –0.1, dropped ears in noninoculated material = –0.1, second-generation European corn borer rating = –0.2, and resistance to root pull = 0.3. Although significant, the correlations were small in all instances. Other traits measured included stand, ear height, ratio of ear height to plant height, nodes with brace roots, nodes with functional brace roots, root and stalk lodging in plants inoculated with stalk-rotting organisms, root and stalk lodging in plants not inoculated with stalk-rotting organisms, tillers (1989 and 1990 only), stalk diameter, Tc (a measure of the stalks resistance to crushing), and first-generation European corn borer (ECB). Thomas-Compton et al.[86] also reported correlations between popping expansion and agronomic traits. Agronomic traits evaluated included yield, stalk lodging, ears per plant, and moisture in the testcross material; yield, moisture, stalk lodging, and dropped ears in two populations; and stalk lodging and sample weight in two populations. The data also included two types of popping expansion data, from an oil popping method and a microwave bag popping method. In all instances, the correlations were low, with the greatest significant correlations occurring between microwave popping expansion and yield (0.44) and moisture (0.34) in the Nebraska population Supergold × Tom Thumb II. Two cycles of S_1 recurrent selection in BHPXD-1C2 for improved expansion also suggested no correlation of popping expansion with agronomic traits.[87] In an evaluation of selection progress in three dent corn by popcorn composites, Spiess[88] reported that while significant gains in popping expansions were made, only yield and stalk crushing strength decreased. No significant changes were observed for percentages of stand, root lodging and stalk lodging, ear height, first-generation ECB rating, second-generation ECB tunnel numbers and length, rind penetrometer resistance, and vertical root pulling resistance. Contrary to the experience gained in working with and growing hybrids, data on segregating lines suggest that gains in expansion can be made without decreases in agronomic performance.

D. Agronomic Traits

Because breeding techniques and inheritance information related to improvement of agronomic traits of popcorn are the same as for the improvement of these traits in field corn, general procedures

and techniques will not be discussed. Minor adjustments to these techniques and procedures that a popcorn breeder must be aware of because of the unique differences between dent corn and popcorn will be reviewed.

A portion of past breeding efforts to improve popcorn's agronomic traits has involved the incorporation of dent corn germplasm into popcorn germplasm. The differences between these two genetic sources necessitate the commitment of a large number of years for this breeding approach. A review of the early literature on dent × pop crosses was given by Robbins.[58] Crumbaker et al.[50] and Johnson and Eldredge[51] discussed dent × pop crosses and suggested that popping expansion could be recovered relatively quickly in a backcrossing program with dent corn as the nonrecurring parent. They suggested that at least one or two backcrosses were necessary to recover acceptable popping expansion. The dent source used also had an effect on how quickly acceptable popping expansions could be recovered. Popping expansion volumes at this time, however, were much smaller than they are today. In these early studies, expansion was the only quality trait discussed, with no reference to other quality traits, except that they were positively correlated.[8] Freedom from hulls, a major quality trait, has undergone improvements, especially in yellow-kernel popcorn, making acceptable amounts of hulls today much less than they were years ago. The trait, freedom from hulls (good hull dispersion),is a challenge to popcorn breeders who want to utilize dent corn germplasm.

Robbins and Ashman[89]examined the use of other corn germplasm to improve the agronomic traits of popcorn and compared the incorporation of dent corn and flint corn into popcorn. Their conclusions supported earlier studies that recovery of expansion is possible. In their study, however, F_2 and F_3 materials, and not backcross material, were evaluated. They concluded that selection in the F_2 generation for popping expansion should be successful with moderate selection intensity to maintain improved agronomic traits. They also concluded that recovery of popping expansion was about the same for the flint as for the dent sources of germplasm, so the choice of germplasm should place more emphasis on agronomic traits than on kernel hardness traits. Similar to earlier researchers, they also concluded that some specific combinations worked better than others. HP72-11, with partial dent components, has been used extensively in the popcorn industry. HP72-11 is from dent × pop material that had undergone two backcrosses to popcorn.[90]

One approach to incorporate dent corn into popcorn being used in the Iowa State University popcorn population development and improvement program is to make the initial dent × popcorn crosses and allow random mating for three to five generations in the cross population (50% popcorn and 50% dent corn), with light selection pressure for harder endosperm kernels. The population at this stage is backcrossed to a dent sterile popcorn to incorporate the dent sterility gene, $Ga1^s$, into the population. Another approach used in the Iowa State University program is to split the initial cross population into two subpopulations. One subpopulation is backcrossed to dent sterile popcorn. The cross and backcross subpopulations are maintained for two to three generations of random mating, with a greater selection intensity for agronomic traits in the cross subpopulation and a greater selection intensity for expansion traits in the backcross subpopulation. The two subpopulations are then crossed using the dent sterile backcross population as the female to incorporate dent sterility into the population. In this last cross of the procedure, *ga*-gametes are lost, with the accompanying loss of any improvement in agronomic traits linked to the *ga* gene from the dent source. The two to three generations of intermating within each subpopulation are necessary to break up linkage blocks before the two subpopulations are merged. The latter approach resulted in the release of BSP3C1 and BSPM2C1.

Breeding for improved yields of popcorn involves the same procedures and techniques as breeding for improved yields of dent corn. The only difference is that, for popcorn improvement, yield is not the primary trait of selection. If a popcorn hybrid does not produce large expansion and good quality flakes, it will not be used in the industry no matter how much it yields. Improved yield of hybrids, though, is an important trait demanded by popcorn growers and, therefore, should be an integral part of any popcorn breeding program. To a lesser extent, improved yields of inbred

lines are of interest to popcorn seed producers. Poor yields of popcorn inbred lines is one of the reasons some popcorn hybrids today are still three-way crosses. It has been observed that, in certain genetic material, intense selection pressure for popping expansion on a single-ear basis in narrow-genetic base germplasm (inbred line development) can result in kernel abortion (poor ear fill). Attention to this detail may help improve yields of inbreds. Any gains a popcorn breeder can make in improved hybrid and inbred yields are welcomed as long as they are not at the expense of popping expansion and quality.

Standability as a trait describes the ability of a popcorn plant to remain erect until the crop is harvested. A main factor differentiating popcorn standability from dent corn standability is that popcorn must stand in the field longer after physiological maturity than dent corn. Popcorn is generally harvested around 16% moisture, whereas dent corn is usually harvested before it dries to 20% moisture. The popcorn plant must remain standing even after the stalk has begun to deteriorate. Two other factors that differentiate popcorn standability and dent corn standability tend to offset each other in their effects. Popcorn ears weigh less than dent corn ears, but generally are located higher on the plant. A popcorn breeder wanting to improve standability might select for lower ear placement and collect standability data after the crop has dried below 16% moisture. In a popcorn breeding program, standability is generally measured by collecting data on root lodging, stalk lodging and, in some instances, dropped ears. Data on root lodging are collected by counting the plants in a plot that are leaning more than 30° at the base of the plant where it enters the soil surface. If root lodging occurs early in the season, the plant will "gooseneck" (start to grow upright again and have a curved stalk); if it occurs late in the season, the whole stalk will remain straight, but at an angle to the ground. Stalk lodging refers to the actual breakage of the stalk at or below the ear. This can either be a clean break in which the ear ends up completely on the ground, or the stalk kinks below the ear, somewhat like what a pipe would do if it were bent. The trait, dropped ears, refers to ears that have fallen off the plant because of a poor shank connection at the base of the ear or a weak or damaged shank. Standability is an important trait in popcorn for many of the same reasons it is important in dent corn; reduced yield losses, ease of harvesting, and more uniform grain drydown. But good standability is of more importance in popcorn than in dent corn. Maintaining minimal damage to popcorn kernels during the harvesting procedure is critical to harvesting a high-quality crop. If plants are severely lodged, combine ground speed must be slowed. This results in the combine not shelling at optimum capacity, increasing the possibility of kernel damage. The ears on stalk-lodged plants that are kinked will be pulled into the combine and shelled. With popcorn's relatively high ear placement, these ears may have been lying on or very near the ground with their quality deteriorating and they will likely have greater moisture contents than ears on erect plants. Both of these factors contribute to decreased quality of the overall harvested crop. Of course, the ears that are lying completely on the ground because of ear droppage or stalks that have broken completely off will not be harvested, reducing the yield.

There are many factors that affect standability of popcorn: mechanical strength of the stalk and roots, high ear placement, susceptibility to the second-generation European corn borer, susceptibility to stalk and root rotting organisms, susceptibility to corn rootworms (*Diabrotica* spp.), and different cultural practices. Popcorn germplasm's susceptibility to the second-generation European corn borer is one of the major factors to overcome in improving popcorn's standability.

The stalks and roots of popcorn hybrids are relatively weak compared with dent corn hybrids. This is because the breeding germplasm of popcorn is weaker than the breeding germplasm of dent corn, a result of the large differences in efforts that have been expended toward the improvement of this trait in dent corn compared with popcorn. Breeding procedures and techniques used for improvement in the mechanical strength of popcorn stalks are the same as used for improvement of dent corn stalks.[91,92] The difference that a popcorn breeder must remember is that the later harvest of popcorn means that popcorn breeders not only need to improve the mechanical strength of the living stalks, but also need to improve the mechanical strength of stalks that are dead and starting to deteriorate.

Root growth of popcorn is not as extensive as that of dent corn. Increased root growth (root strength) could help improve popcorn germplasm's resistance to root lodging, which results strictly from poor root development. The same selection procedures and measuring techniques used to improve the roots of dent corn also can be used to improve roots of popcorn — some evaluation of root pulling resistance.[93] Another trait that may play a role in improving popcorn's resistance to root lodging is the development of adventitious roots. Popcorn generally has few, if any, adventitious (brace) roots; dent corn has many. Perhaps selecting for increased numbers of adventitious roots could provide for increased stability of the popcorn plant at ground level.

The same insects that infest dent corn also infest popcorn. The most damaging of the soil insects are the northern corn rootworm (*Diabrotica longicornis barberi* Smith & Lawrence) and the western corn rootworm (*Diabrotica vergifera vergifera* Leconte).[15] A screening of popcorn germplasm in 1985 by the USDA Insect Research Laboratory near Brookings, SD (Iowa State University, unpublished data) showed no resistance to the western corn rootworm in the materials evaluated. Rootworm larvae feed on the roots and, if the infestation is large enough, can damage the roots so severely that the plants lodge. This lodging can have a detrimental effect on the quality of the crop harvested from the field. The adult rootworm beetles emerge in the summer and feed on silk, pollen, and ears. Generally, breeding for resistance to corn rootworms has been based on evaluation under natural infestation, but progress has been poor. Although there are methods available for artificial infestation,[94] they are not actively used in most popcorn breeding programs because of the resources required. Some other soil insects that can pose severe problems to popcorn are wireworms [*Agriotes mancus* (Say), *Horistonotus uhlerii* Horn, *Melanotus cribulosus, Aeolus mellillus*, and *M. communis*], grubs (*Phyllophaga* spp.), and cutworms [*Agrotis ipsilon* (Hugnagel), *Feltia ducens* Waker, *Crymodes devastator* (Brace), *A. gladiaria* Morrison].

The most destructive insect pest of popcorn is one of the foliar- and stem-feeding insects, the European corn borer (ECB). The ECB poses a much greater threat to popcorn than dent corn because there is less tolerance to this pest in popcorn germplasm. In most popcorn production areas, there are generally two, and sometimes three, generations of ECB that attack popcorn in one growing season. Resistance to the first-generation ECB is under different genetic control than resistance to the second-generation ECB.[95] Unpublished data collected in the Iowa State University popcorn breeding program have shown that popcorn germplasm generally has slightly more tolerance to first-generation ECB than to second-generation ECB. Even a very light infestation of second-generation ECB can result in economic loss when tolerance to the ECB is low.[26] Breeding schemes and selection procedures for resistance to both generations of the ECB in popcorn are the same as those used in dent corn breeding programs. If the use of genetically modified organisms is accepted by popcorn consumers, the incorporation of *Bacillus thuringiensis* (Bt) into popcorn can assist in the control of damage by the ECB. In 1999, the Iowa State University program released BSP6CBC0, a South American type popcorn synthetic with a higher level of natural tolerance to the second generation ECB than other popcorn germplasm.

Damage by ECB feeding affects popcorn in many different ways. ECB damage to popcorn not only affects agronomic traits as it does in dent corn, but it also affects the quality traits of popcorn.[96] In one study, Jarvis et al.[26] showed no effect of ECB infestation on expansion, but a later study by Jarvis et al.[97] showed a decrease in popping expansion related to increased feeding of the ECB. Second-generation ECB will also feed on the ear, causing kernel damage. Resistance in popcorn germplasm to the second-generation ECB most likely can be traced back to the incorporation of dent corn germplasm.

Another foliar- and stem-feeding insect that causes damage to popcorn in some years is the corn earworm [*Helicoverpa zea* (Boddie)]. Because any damage to the pericarp of a popcorn kernel reduces potential popping volume, a large infestation of corn earworm can reduce popping quality. Also, corn earworm feeding sites allow for infestation by kernel rots and other ear diseases. Unlike resistance to the second-generation ECB, resistance to corn earworm has been found in popcorn germplasm.[98–100] With this resistance available in popcorn germplasm, breeding for resistance to

this pest should be easier because nonpopcorn germplasm (with its associated loss in quality) will not need to be used as a source of resistance.

Other foliar- and stem-feeding insects that attack popcorn are aphids [*Rhopalosiphum maidis* (Fitch)], armyworms [*Pseudaletia unipuncta* (Haworth)], mites [*Oligonychus pratensis* (Banks)], and grasshoppers (*Melanoplus* spp.). Under unique growing conditions, any one of these pests, and others not mentioned, may pose a severe problem in a local growing area. Generally, though, they pose less of a problem over the entire popcorn growing area and are not routinely dealt with in popcorn breeding programs.

Stored-grain insects also can pose a serious problem for the popcorn breeder. There is no information available on the resistance of popcorn germplasm to stored-grain pests, but it is best to assume all are susceptible. Most control measures include environmental (storing at 0°F or colder for 24 hours or more[101,102]) or chemical control. A popcorn breeder must read insecticide application labels carefully and use only those approved for use on popcorn.[103]

Susceptibility of popcorn plants to insects also can aid infection by diseases. Insect feeding sites provide wounds for entry by disease-causing agents. A study by Jarvis et al.[97] has shown that susceptibility to ECB in popcorn can increase severity of damage by stalk rots. The opposite also can occur where insects do better on plants that are diseased, such as ECB larvae developing faster on plants infected with anthracnose [*Colletotrichum graminicola* (Ces.) G.W. Wils.].[104]

All diseases that attack dent corn also can attack popcorn. The severity of diseases affecting popcorn fluctuate according to environmental conditions, susceptibility of germplasm, and the presence or absence of disease-producing organisms. Stalk and root rots, however, cause the greatest loss in yields and quality in popcorn for the entire popcorn growing area. Stalk and root rots are caused by several fungal and/or bacterial pathogens rather than by a single causal agent.[15] The actual makeup of the complex of pathogens varies among locations and years, so a popcorn breeder considering artificial inoculation needs to have some knowledge of the causal agents in popcorn growing areas. Popcorn breeders who do not use artificial inoculation rely on natural inoculation in nurseries to assist in selection for resistance. Yield losses occur as a result of infected plants having poorly filled ears and/or lodged stalks. Quality losses occur from reductions in standability and, in severe instances, from premature death of the plant, resulting in poorly formed and filled kernels.

Severe infections by ear and kernel rots can have a devastating effect on quality, because popcorn is used for human consumption and because diseased kernels do not pop well, if at all. Because of its much thicker pericarp and harder starch, popcorn generally seems more resistant to ear diseases than other types of corn, but within popcorn germplasm differences in *Aspergillus flavus* infection have been reported.[105] From a popcorn breeder's standpoint in selecting for resistance, all diseased ears are discarded. Popcorn kernels also are susceptible to a storage disease referred to as blue-eye condition. Bullerman[106] and Nelson[107] discuss this disease and state that the molds isolated from diseased kernels were mainly *Penicillium* spp. Katta et al.[108] provide listings and discussions of other storage molds that can be found in stored popcorn. Katta and Bullerman[109] report a three-way interaction between spore inoculum, time of storage (at high temperatures and humidities), and popcorn genotype. This suggests that popcorn breeders might be able to breed for improvement in popcorn's tolerance to stored grain molds under certain storage conditions.

The leaf spots and leaf blights that attack dent corn also attack popcorn. Some of these diseases have been studied in popcorn. (1) Northern corn-leaf-blight [*Exserohilum turcicum* (Pass.) Leonard and Suggs]: The *Ht1* gene that conditions a chlorotic lesion type of resistance to this disease was found in a popcorn variety — Ladyfinger.[110] In a summary of northern corn-leaf-blight resistance in popcorn germplasm, Ashman[111] lists nine popcorn lines that carry the *Ht1* gene (A1-6, IDS53, HP62-52, HP68-07, HP301-Ht, HP62-02Ht, IDS69-Ht, SG1533-Ht, and 4722-Ht). (2) Eyespot (*Kabatiella zeae* Narita and Hiratsuka): After a 1981 outbreak of eyespot in Iowa, 400 popcorn entries were evaluated for resistance. Results summarized by Ziegler[112] indicate that popcorn germplasm seems to be somewhat resistant to eyespot. (3) Goss's bacterial wilt and blight disease

[*Clavibacter michiganense* spp. *nebraskense* (Schuster, Hoff, Mandel, and Lazar) emend. Vidaver and Mandel (Syn. = *Corynebacterium nebraskense*)]: Causal organisms have been used to screen popcorn germplasm for resistance to this disease, which occurs mainly in certain parts of Nebraska. Under natural inoculation conditions, Wysong et al.[113] screened 16 popcorn inbred lines and 38 popcorn hybrid varieties. The average disease intensity for inbreds was 2.9 (rated on a scale in which 0 = no disease to 6 = dead plants). The range for hybrids was 1.5 to 5.0. (4) Headsmut fungus [*Sorosporium reilianum* (Kühn) McAlp]: A severe head smut infection in a Nebraska popcorn field in 1989 caused by *S. reilianum* prompted Wysong to initiate an evaluation of this disease's effect on popcorn.[114]

This listing of diseases and insects does not include all of the diseases or insects affecting popcorn, but includes those for which references were found. A popcorn breeder's goals, as they pertain to diseases and insects, are to identify and select for resistances to those pests that pose the greatest economic and quality losses to popcorn. After considering the resources available for a breeding program, a popcorn breeder must then determine where to allocate those resources by deciding how important pest resistance is to the overall program and then which specific pests to emphasize in selection.

E. Other Traits

Dent sterility is an important trait in any popcorn breeding program. Nonreciprocal cross incompatibility systems between different types of *Zea mays* have been known for many years. Whitely et al.[115] provide a review of the early literature on this subject specifically as it pertains to popcorn. Other reviews of the literature on nonreciprocal cross incompatibility systems in *Zea mays* are provided by Sukhapinda[116] and Kermicle and Allen.[117]

Early literature indicated that not all popcorn germplasm carried this genetic mechanism for preventing pollination by dent corn. Thomas[118] describes a method for incorporating this genetic mechanism into popcorns that do not carry incompatibility genes. In popcorn, this incompatibility system is attributed to the effect of an allelic system of the *Gal* locus, with Gal^s being the most prominent allele used in popcorns. Presently, it is almost a necessity for new popcorn inbred lines to be homozygous for Gal^s. The main reason is that the majority of hybrid popcorn seed is produced in areas where dent corn is grown and, even under the best isolation conditions, dent corn pollen can get into a popcorn seed production field. If the popcorn plants are receptive to dent pollen, the result is popcorn × dent corn F_1 plants growing in commercial popcorn fields. The seed from F_1 plants has very poor popping quality and, if present in a large enough frequency, can decrease the popping quality of the harvested crop. If the crop is ear picked, F_1 ears can be removed by hand sorting; if the crop is combine harvested, however, it can be very difficult to sort out the F_1 kernels.[15]

Because of the possibility of contamination in seed production fields, a popcorn breeder needs to strive toward developing only dent-sterile inbred lines. In an effort to do this, the Iowa State University popcorn breeding program has developed a series of different maturity dent corn stocks that carry purple aleurone genes to use as testers for dent sterility. The purple aleurone dent pollen is used to pollinate a popcorn ear on one day, and the next day that same popcorn ear is self-pollinated. At harvest time, any of the ears containing purple kernels are discarded. This is done at the S_1, S_2, and a later generation of selfing to identify heterozygous lines and escapes. If the source population of the inbred lines is homozygous dent-sterile for Gal^s, all lines from the source should be dent sterile. But, some popcorn germplasm is more difficult to convert completely to dent sterility than others. If there is any doubt about the dent sterility of the source population, dent sterility should be checked at least once during the later generations of an inbred's development.

For commercial popcorn production fields, from which the harvested crop is to be used for popping, there is little need for isolation from dent corn, even if the popcorn hybrid is not dent sterile. Though some xenia (immediate effect of pollen on a corn kernel) does occur, reducing individual kernel popping expansion when the pollen source is not popcorn,[54,119] there is so much

popcorn pollen available in a commercial popcorn production field that frequencies of outcrossed kernels are low in the total harvested crop, rendering the effect nonmeasurable.

Nearly all popcorns are prolific (i.e., popcorns have more than one ear per plant). Some of the more prolific popcorns are the novelty popcorns, which can have up to six ears per plant under the right growing conditions. Prolificacy in popcorn has advantages and disadvantages. Two well-developed ears per plant can provide increased yields. If germination is poor or if anything occurs to reduce stands, prolific hybrids produce additional ears per plant. On the negative side, maturity of the ears is generally not the same, which lengthens the maturity window for popcorn to escape an early frost and also makes determining optimum harvest time more difficult. When making selections, popcorn breeders must decide for their own programs whether to consciously select for or against prolificacy or not be concerned with it at all and let their germplasm and other selected traits determine prolificacy.

Ear placement on popcorn generally tends to be two to three nodes higher than in dent corn. Popcorn breeders should emphasize lower ear placement in the breeding nursery. Lower ear placement lowers the center of gravity of the popcorn plant, which can help to improve standability.

Literature reviews on the potential uses of molecular markers have been discussed by several authors.[120–132] A knowledge of the location of molecular markers relative to genes controlling traits important in popcorn improvement can be beneficial to popcorn breeders. Stuber and Goodman[133] included 22 popcorn lines in their allozyme characterization of 406 U.S., Canadian, and European inbred lines of corn, and found allelic differences at 12 of 22 allozyme loci in the popcorn lines evaluated. Bretting and Wendel[134] evaluated seven popcorn inbred lines and reported allelic variation at *Tpi3* and *Tpi4* loci, with no variation at *Tpi1*, *Tpi2*, or *Tpi5*. Isozyme data (Table 7.3) also indicate variation within and among enzyme loci in popcorn. Kahler has observed differences at 19 loci on public and private popcorn germplasm.[135]

Thomas-Compton reported use of a mutant form of *Adh2* in IDS69 as a tool for identifying dent-sterile progeny.[136] The *Adh2* locus is "loosely linked" to the $Ga1^s$ (dent-sterile) locus. Thomas-Compton also has found an association of a thick, double protein band in the 38kD region from pollen samples of dent-sterile genotypes.[137] Normal genotypes (both non-dent-sterile lines of popcorn and dent corn lines) did not possess the lower and stronger band of the two. Using inter-simple sequence repeat (ISSR) amplification, Kantety et al.[138] were able to classify 19 popcorn and 8 dent corn inbred lines into their correct heterotic groups. Senior et al.[139] included a few popcorn inbred lines in their simple sequence repeat (SSR) study on relationships in maize and were able to accurately separate all the popcorn inbred lines but one. Knapp[140] and Xie and Xu[141] provide discussions on the incorporation of molecular marker assisted selection into breeding strategies. The actual use of molecular markers in a popcorn breeder's program will depend upon the resources and objectives of each individual breeding program.

In 1998 and before, there were no genetically modified commercial popcorn hybrids available. With the success of incorporating *Bacillus thuringiensis* (*Bt*) and herbicide resistance into dent corn germplasm using genetic engineering and transformation techniques, the further incorporation of these traits into popcorn will occur. The acceptance of use of this technology to improve popcorn can provide popcorn breeders access to sources of traits that had not been available before. However, the extent of use of this technology to incorporate new traits into commercial popcorn hybrids is dependent upon consumer's acceptance of genetically modified traits in their food.

VII. FUTURE OUTLOOK

The microwave popcorn industry has provided consumers with a clean, convenient snack that can be prepared wherever there is a microwave. The prepopped-popcorn industry, which includes the flavored, candy coated, and normal popped flakes, has taken a different approach to make popcorn available in another form to millions of consumers. New popcorn poppers are constantly being designed and built to aid the home consumer in preparing popcorn in the more traditional way.

TABLE 7.3
Observed Allozyme Variation Within and Among 19 Enzyme Loci in 43 Popcorn Inbred Lines Maintained at Iowa State University, Ames, IA (Biogenetic Service, Inc. Nomenclature)

	Enzyme locus																		
Entry	Adh1	Acp1	Acp4	Mdh1	Mdh2	Idh2	Pgd1	Pgd2	Glu1	Pgm2	Phi1	Amp1	Amp3	Enp1	Est1	Est4	Got1	Got2	Px1
IDS38W	2	1	2	2	2	2	1	1	1	3	2	1	3	1	2	3	2	1	1
IDS43W	2	1	1	2	2	2	1	1	1	3	2	1	3	3	2	3	1	1	2
HP303W	2	1	2	2	1	2	1	1	1	2	2	1	2	1	1	3	2	1	1
HP304W	2	3	5	2	1	1	1,2	1,2	1	3	2	1	2	3	1	3	1	1	1
I5	1	1	2	2	2	2	1	1	1	2	2	1	3	1	2	2	1	1	2
I12	2	1	2	2	1	2	1	1	1	3	2	1	2	3	2	2	1	1	2
I27	2	1	1	2	2	2	1	1	1	3	2	1	3	3	2	3	1	1	1
I29	2	3	1	2	1	2	1	1	1	2	2	1	2	1	2	4	2	1	2
I56	1	3	5	2	2	2	2	1	1	2	2	1	2	3	2	3	1	1	2
I62	2	3	1	2	1	2	1	1	1	2	2	1	2	3	2	3	2	1	1
IDS70	2	2	1	2	2	1	2	1	1	2	2	2	1	3	2	3	1	1	2
IDS91	2	3	2	2	1	2	1	1	1	2	2	1	3	3	2	3	2	1	1
IDS69	2	3	2	2	1	2	1	1	1	2	2	1	3	3	2	3	2	1	2
I69	2	3	1	2	1	2	1	1	1	2	2	1	3	3	2	3	2	1	2
HP301	2	3	2	2	1	2	1	1	1	2	2	1	3	3	2	3	2	1	2
IDS28	2	3	1	2	2	2	2	1	1	2	2	1	2	3	2	3	2	1	2
SA24	2	3	2	2	1	2	1	1	1	2	2	1,2	1,3	3	2	3	1,2	1	1,2
SA1490	2	3	2	2	2	2	2	1	1	2	2	2	3	3	2	3	1	1	2
SG1533	2	3	1	2	2	2	1	1	1	2	2	1	3	3	2	4	2	1	2
SG18	2	3	1	2	2	2	1	1	1	2	2	1	3	3	2	4	2	1	2
4619-33	2	3	1	2	1	1	1	1	1	2	2	1	3	3	2	4	2	1	1
SG30A	2	3	1,5	2	1,2	1,2	9	1	1	2	2	1,2	3	3	2	4	2	1	1

SG32	2	3	1	2	2	2	1	1	1	2	2	1	3	3	2	2,4	2	1	2
SG16	2	3	1	2	2	1,2	1	1	1	2	2	1	3	3	2	4	2	1	1
4722	2	1	2	2	1,2	2	2	1	1	2	2	1	3	3	2	3	2	1	1
HP62-08	2	3	2	1	1	2	2	1	1	2	2	2	2,3	3	1	3	1	1	1
HP62-15	2	3	1	2	1	2	1	1	1	3	2	1	3	3	1	3	2	1	1
HP62-49	2	3	2	2	2	2	1	1	1	2	2	2	2	3	2	4	2	1	2
HP68-07	2	2	1	2	2	2	1	1	1	2	2	1	2	3	2	3	2	1	1
HP72-11	2	3	2	2	2	2	1	1	1	2	2	2	3	3	1	3	2	1	1
P1	2	2	5	2	2	2	2	1	1	2	2	1	2	3	2	2	2	1	2
P5-BA	2	2	5	2	2	2	2	1	1	2	2	1	2	3	2	3	2	1	2
P18	2	1	2	2	1	2	1	1	1	3	2	1	3	3	2	2	1	1	1
P40	2	1	1	2	1	1	2	1	1	3	2	2	3	3	2	4	1	1	2
C-6-29	2	1	1	2	1	2	1	1	1	2	2	2	2	1	2	2	1	1	2
C-1-29	2	1	2	2	1	2	1	1	1	3	2	1	2	1	1	2	1	1	2
A1-6	2	3	2	2	2	2	1	1	1	2	2	1	2	3	2	3	2	1	1
KP47	2	3	5	2	1	2	2	1	1	2	2	2	3	3	2	3	2	1	1
KP48	2	2	1	2	2	1	2	1	1	2	2	2	1	3	2	3	1	1	2
KP58	2	3	2	2	1	2	1	1	1	3	2	1	3	3	1	3	2	1	2
N42	1	3	2	2	1	2	1	1	1	2	2	1	3	3	2	3	2	1	1
NP86	2	1	2	2	2	2	2	1	1	2	2	1	2	3	1	4	2	1	2
NP87	2	3	1	2	2	2	2	1	1	2	2	1	2	3	2	5	2	1	2

Note: Translation of information is available from Biogenetic Services, Inc., Brookings, SD 57006.

New, unique uses and techniques for utilizing popcorn are being thought of and developed every day. With consumer acceptance, new genetic engineering techniques can provide popcorn growers and consumers with better performing popcorn hybrids and better tasting and nutritionally more healthful popcorn.

The demand for snack foods is high. In 1998, snack foods represented a 43.5 billion dollar U.S. industry.[12] The formation of a Popcorn Promotion Board should increase popcorn's share of the snack food industry's dollars. Consumption of popcorn during the past several years has been steadily increasing at an approximate annual rate of 4 to 5%.[142] Traditionally, popcorn has been the leading specialty corn item exported from the U.S.[143] For a recent span of seven years, in thousands of metric tons, these amounts are as follows: 1992, 116; 1993, 130; 1994, 126; 1995, 131; 1996, 122; 1997, 96; and 1998, 99.[144] Over those 7 years, U.S. popcorn export income averaged $70 million a year. More countries are experimenting with producing popcorn. If they are successful, U.S. popcorn exports may decline, but the overall world consumption of popcorn could increase significantly as more people worldwide get introduced to eating popcorn. The possible uses of popcorn and new marketing strategies are limited only by the imaginations of people working with and eating popcorn, providing a constant challenge to the popcorn breeder to meet the continually evolving demands placed on popcorn.

VIII. SUMMARY

Popcorn has been a commercial commodity crop in the U.S. for more than 100 years. During this time, popcorn has proven to be more than just a "fad" commodity. One reason is the popcorn industry's willingness to make changes to keep pace with the ever-changing demands of a constantly changing and developing society. This willingness of the popcorn industry to adapt to change, and at times even be the leading force behind change, provides an interesting challenge to the popcorn breeder. A successful popcorn breeder will remain informed of, and at times take a leading position in, the constant evolution of the popcorn industry. Fifteen years ago, few, if any, popcorn breeders were concerned with microwave popping popcorn. Today, a large portion of the popcorn produced is used in the microwave popcorn industry. Popcorn breeders have developed popcorn hybrids and germplasm that not only meet the demands of oil popping, but also meet requirements for performing well under microwave popping procedures. Popcorn is a specialty corn; the popcorn industry is composed of a number of specialty users. Popcorn breeders must allocate resources wisely to the areas where each individual breeding program can provide its greatest impact toward the improvement of popcorn. Each new popcorn hybrid represents a compromise among all the traits discussed in this chapter and provides unique, usually small increments toward the continuing goal of improving this specialty corn, popcorn.

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8 Breeding White Endosperm Corn

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I. INTRODUCTION

A. General Description of White corn

A simple description of white corn is corn grain without yellow pigmentation. The white color is understood to be controlled by a single recessive gene, *y*, where dominant *y* gives yellow color. Although perception is true, the modern user of white corn demands a product from the farmer's field that is more precisely defined. The endosperm of the grain must be pure white, not only devoid of yellow pigments, but also red or blue colors caused by anthocyanin pigments and brown or dingy discolorations that are assumed to be caused by other flavonoid compounds. The aleurone layer and pericarp must be clear, also devoid of anthocyanin and other flavonoid compounds. The embryonic axis and scutellum should not be pigmented and the cob should be white (colorless). Many genes control the presence or absence of pigmentation or discoloration and some can seriously complicate the development of new white corn varieties. In addition, the most desirable white corn grain has a large uniform kernel, has no or only a slight dent, has a high specific gravity, has no stress cracks, and is free of kernel rots, particularly those that cause accumulation of aflatoxin. Many historically important varieties and modern hybrids achieve the high standards listed above. However, the introduction of new genetic sources into a white corn breeding program, such as crosses with elite yellow inbreds or even unrelated white inbreds, may introduce genes that can complicate the recovery of the ideal white endosperm genotype. This complication has always plagued white corn breeding and will continue to do so in the future. An understanding of the possible genetic complications will be discussed later to help the white corn breeder understand and possibly avoid dead-end efforts.

B. Importance of White Corn in the United States

Yellow endosperm corn dominates U.S. corn production, but white corn has played an important role in the history of corn and continues to be a significant U.S. agricultural commodity. Native American civilizations utilized many color variations of corn and several endosperm variations. White or white with red, blue, or purple accents were often preferred because of religious significance, and flint, floury, sugary, or dent types were favored because of the method of grain preparation or utilization. Other selections may have been based on taste or palatability. Early colonists acquired the seedstocks and production methods from the native Americans, began utilization of corn for food, and started adaptation of seedstocks without considering color variation. Later modifications tended to stabilize color variation so that in 1899, most open-pollinated varieties were predominantly either yellow or white. About 49% of the 507 open-pollinated populations described by Sturtevant[1] were predominantly white. By 1936, State Agricultural Experiment Station researchers had evaluated many of the open-pollinated varieties and recommended the best for use in their states. According to Jenkins,[2] about 37% of 383 recommended varieties had white germplasm. A preference had developed for white corn types in the southern and western Corn Belt, although white endosperm as well as yellow tryptophan varieties were often recommended for the central and northern states. The most significant determinant for the domination of yellow over white endosperm came with the discovery that white corn lack the vitamin A activity of yellow corn; thus, it was less desirable as a feed for animals. A preference for the taste of white corn products for human consumption, however, continued.

C. White Corn Production: Quantity and Location

Production of white corn in the U.S. from 1917 to 1987 was summarized by Thomas.[3] Excerpts of her summary are shown in Table 8.1. In 1918, the production of yellow and white corn was about equal. During the 70-year interval that followed, white corn production declined significantly while yellow corn production increased dramatically. From 1986 to 1987, U.S. white corn production was only about 711 thousand metric tons (28 million bu), less than 1% of the yellow corn production. The reduced production can be related directly to decreased use as a feed grain. The area of production also shifted during this time interval, and the changes can be related to utilization. From 1917 to 1918, the production of white corn was primarily in the central U.S. Corn Belt (Table 8.1) where the largest acreages of corn were being grown and where utilization was primarily as a feed grain. White and yellow corns were used interchangeably. When white corn was no longer desired as an animal feed, the major utilization became traditional human food rather than animal

TABLE 8.1
Production of White Corn in the U.S. from 1917 to 1987

Year	Total U.S. production (1000 bu)	States with most production in descending order	Largest state production (1000 bu)
1917–1918	1,076,494	IL, IN, IA, MO, NE	141,000
1918	(1,077,607)		
1942–1946	464,759	TN, AL, GA, NE, NC	40,488
1942–1946	(2,686,686)		
1972–1976	39,299	KY, TN, GA, TX, MO	9781
1975	(5,754,402)		
1986–1987	28,221	TX, KY, TN, MO, IA	8540
1986–1987	(7,630,266)		

Note: Numbers in parentheses are for yellow corn production in the year or time period indicated in column 1.

feed. By 1942 to 1944, white corn production became concentrated in the south where a significant amount of corn was utilized for human food as grits, hominy, and corn flours. White corn was generally preferred over yellow. From 1972 to 1976, and then from 1986 to 1987, the principal production moved to other areas of the south and then west. Large dry-milling plants and shipping facilities were located in or near the Ohio and Mississippi river valleys. Until the late 1980s, when some of the larger dry-milling plants were closed, the processing plants and shipping facilities served as a magnet to draw white corn production into the local area. A separate factor may have been of greater significance for the westward move of white corn production. Use of white corn grew during the late 1970s and 1980s for ethnic foods and snacks such as tortillas, tortilla chips, and extruded products. Much of the consumer demand and processing facilities were centered in the southwest and west; thus, production increases in Texas and California. In addition, white corn grain exports to Mexico increased and southwest production was convenient. Irrigated production of white corn on the high plains of Texas also contributed to the westward movement of white corn production since it allowed high grain yield, rapid field dry-down, and high quality grain production.

Price per bushel for white corn generally exceeds that for yellow corn. Since 1971, the Kansas City, MO price per bushel has been the same for white and yellow corn grain only in 1972 and 1986, whereas the advantage for white corn for ranged from just a few cents to about $1.75 per bushel in other years.[3] Declining or variable supply with a relatively constant demand contributed to the need for a price bonus for white over yellow corn. Part of the variable supply occurred because farmers were often hesitant to grow white corn because they considered it to have lower yield potential and greater susceptibility to production hazards. Until recently, white corn has been marketed through local grain elevators or other intermediaries rather than by direct contract production with the grain processor. In recent years, contract production of white corn as a specialty crop has become more prevalent, particularly as processor needs become more specific and demanding.[3]

D. Special Production Considerations for White Corn

The management of a white corn grain crop must emphasize high quality grain. Management considerations have been reviewed by Josephson and Berggren[4] and Asgrow Seed Co.[5] (anonymous author and date). Some of the major considerations they discuss follow. First, a hybrid should be selected with a maturity that is adapted to the intended area of production. It should have appropriate disease and insect tolerances for local pest problems. A hybrid should have the appropriate kernel type, be resistant to lodging caused by diseases and insects, and should have uniform, large dense kernels on an ear with complete husk cover to discourage entry by insects and birds. Useful hybrid selection guidelines may be obtained from dealer recommendations, Agricultural Experiment Station hybrid performance evaluations, tests conducted by local farm groups, and from experiences of neighbors. A cooperative effort of public and private breeders has been conducted since 1977 to provide regional evaluations, from the far west to the central U.S. Corn Belt to more southern locations, of newly released commercial white corn hybrids (Darrah et al.[6–20]). Second, fertility, herbicide, and insecticide applications should conform to the best recommendations for the production area. Agriculture Experiment Station recommendations may be obtained from the local county agricultural extension agent. Seed company agronomists can also provide useful management inputs. In general, however, management inputs for white corn will not differ from yellow corn except that extra care should be expended to avoid stress from drought or damage from diseases and insects that might contribute to stalk lodging or fungal growth on kernels. Clean grain is most easily removed from the field when excessive weed growth or lodging do not introduce unwanted trash into the harvested grain. Third, harvest at relatively low moisture content. Grain harvested at less than 20% grain moisture and proper adjustment of harvest equipment will help to avoid broken, cracked, or crushed kernels and ensure properly cleaned grain. Trash, such as dirt or weed plant parts or seeds, parts of cobs, and broken or smashed kernels, can provide initial sites for fungal

growth in stored grain. Fourth, proper drying after harvest is essential. White corn should be dried to below 14% moisture as soon as possible after harvest to avoid storage mold growth. Caution should be exercised, however, to avoid rapid drying at high temperatures. Excessive stress cracking may result from too rapid drying conditions and will reduce the value of the white corn grain for many food uses.

II. INHERITANCE OF WHITE ENDOSPERM COLOR IN CORN

A pure white corn produced on white cobs is desired for most processed corn products and particularly for the products of dry milling. Traditional white corn varieties have been developed with excellent white endosperm and white cob characteristics, but crosses with yellow endosperm germplasm or even unrelated white germplasm can introduce unwanted pigmentation. The unwanted pigmentation can accumulate in various kernel parts, endosperm, embryo, aleurone layer and pericarp, glumes, or in the cob. The pigments may be carotenoids (yellow pigments in the endosperm), anthocyanins (red or purple pigments in endosperm, embryo, aleurone layer, pericarp, glumes, and cob), and other flavonoids (brown or dingy off-colors in kernel parts and cob). A large number of genes exert control of the pigmentation even though a single recessive gene, *y,* is the major determinant of white rather than yellow endosperm color. Other loci can mimic the white endosperm appearance of *y,* can modify the white phenotype of *y,* can add other pigments to kernel parts, or can modify cob color. The inheritance of corn pigmentations has been studied extensively and many phenotypes have been explained. A comprehensive listing and description of these genetic studies were presented by Coe et al.[21] A restatement of a portion of their genetic summary plus details provided by other authors, as related to the white endosperm and white cob phenotypes, is presented.

The genetic explanation for the difference between white endosperm and yellow endosperm corn was provided by Correns[22] in 1901, who demonstrated that the homozygous recessive genotype at the *y* locus produced a white endosperm while presence of the dominant allele, *Y,* in the genotype added yellow color to the kernel phenotype. Because the endosperm of corn is triploid, 0 to 3 *Y* alleles may be present in an endosperm genotype and will affect the intensity of the yellow color. The yellow color occurs as a result of the accumulation of carotenoid pigments (carotenes and xanthophylls). The primary carotenoid pigments found in corn grain are beta-carotene, beta-cryptoxanthin, lutein, and zeaxanthin.[23] In addition to providing color, the carotenes are precursors of vitamin A. Xanthophylls provide only half as much or no vitamin A activity compared with beta-carotene since one or both of their terminal ring structures has an –OH group. Although less important for vitamin A activity, the xanthophylls are valuable in poultry feed as they impact a desirable color to egg yolks and skin. Mangelsdorf and Fraps[24] determined that the dominant *Y* allele adds vitamin A activity to corn grain in a linear dosage relationship. Using the nomenclature convention suggested by Coe et al.,[21] this locus for yellow endosperm pigmentation is labeled *y.* Other reports in the literature have referred to this locus as *y1,* which should be considered an equivalent symbol.

The *y* locus is tissue specific in that it results in the absence of all carotenoid pigments and their precursors in the kernel endosperm but does not affect leaf chlorophyll development/accumulation or abscisic acid accumulation in the kernel. Tissue specificity is important in that other loci, which can alter endosperm carotenoid pigmentation to produce white or nearly white kernels, may also reduce carotenoid pigment contents in chloroplasts. Carotenoids are essential to chloroplast membranes, and when absent or modified, can alter normal chlorophyll development or allow photoauto-oxidation of chlorophyll in seedlings.[25] Carotenoids also have a necessary function in absorption of specific wavelengths of light by chloroplasts and can influence the efficiency of photosynthesis.[26,27] In addition to carotenoid effects on photosynthesis, several loci that can alter endosperm carotenoid pigmentation can also cause failure of normal embryo dormancy (vivipary). Vivipary is controlled by levels of abscisic acid,[28–30] which is synthesized via the carotenoid pathway.

Robertson[31] provided a summary of mutants that may produce albino seedlings, with or without white endosperm seeds and viviparous embryos. He classified the mutants into two classes: Class I types are those that are "… characterized by white or pale-yellow endosperm (in a genotype that would otherwise produce yellow seeds) and albino seedlings …" and Class II types are those that consist of "… albino mutants in which pigmentation of the endosperm is not affected." Class II mutants can easily be eliminated by normal breeding procedures owing to the appearance of albino seedlings and yellow endosperm color, but Class I mutants may persist or cause confusion in white endosperm breeding programs because some genotypes with white or pale-yellow endosperm color may have green, pale-green, or mutable alleles that might be overlooked in initial screenings.

The *y* locus is classified as a Class I type and has been shown to have alleles, *y-8549* and *y-w mut,* which produce light green seedlings and are temperature sensitive.[32] However, the standard *y* allele affects only the kernel by total elimination of carotenoid pigments in the kernel endosperm. It is not temperature sensitive, seedlings have normal green coloration, and seeds have normal dormancy. Other Class I loci (*vp2, vp5, vp9, ps, w3, ae,* and *y9*) cause either white or pale yellow endosperm by complete or partial elimination and beta-carotenoid pigment formation and the accumulation of carotenoid precursors, whereas *lw, lw2, y10, (w7748), lw3, lw4, c1,* and *y* do not accumulate beta-carotene or its precursors.[31] Numerous occurrences of white endosperm mutants have been studied and named with symbols that may cause confusion. According to Robertson,[31] "… the *Y* has been used three times (i.e., *Y-d, Y-r,* and *Y5*) and the *y* symbol has been used eight times (i.e., *y, y2, y3, y4, y6, y7, y8,* and *y9*) in connection with the inheritance of yellow endosperm." Of these, "… *y2, y3,* and *y4* are no longer used, and *y6* has been replaced by *Y-r*."[31] The *Y5* factor modifies the white endosperms of *y3(a1)* and *y7(vp9)* to yellow, but *y5* does not directly cause a white endosperm. Therefore, use of this symbol is inappropriate. The *y8* factor, according to Robertson[31] and Graner,[33,34] is allelic to the gene identified as *Y-d,* which dilutes orange endosperm to yellow. The *y2* locus has been shown to be allelic to *vp2, y3* to *a1, y4* to *y* (Graner[34,35]), and *y7* to *vp9* (Robertson[31]). Both *Y-d(y8)* and *Y-r(y6)* diminish carotenoid pigments in the endosperm to produce a pale yellow color.

Pale yellow endosperm can also be caused by the *Bn* (brown aleurone) locus in interactions with *y, y3(a1),* and *y7(vp9),* which cause brown to pale-yellow pigment production in the aleurone.[34,36] Kulkarni[37] also described the production of pale yellow endosperm by the action of *Wc* (white cap) in seeds with the *Y* allele. The dominant factor *Wc* or its allele even when homozygous, will completely eliminate yellow endosperm pigments.[31] *Wc* may modify *y* endosperms to whiter color (E. Coe, personal communication).

Although many loci can cause nearly white to white endosperm, the only acceptable locus and allele to provide white endosperm inbreds is *y* in the absence of modifying loci. Kernels with pale yellow endosperm are not acceptable for white endosperm processed products; albino or pale green seedlings have impaired photosynthetic abilities; viviparous kernels have unacceptable processing quality and seed germination; and nonallelic loci will produce either yellow or off-color progeny in hybrid combination with *y* inbreds.

Red or purple colors, produced by anthocyanins, can occur in the pericarp, glumes, aleurone, embryo, and endosperm of kernels as well as in leaves, roots, glumes, and cobs.[21] These pigments are nonvital but have been useful as markers in genetic studies. Some color combinations had religious significance to American Indian societies and connotations of good luck for early American settlers. Anthocyanins are flavonoid pigments, glycosides of water-soluble phenolic derivatives, that can impart coloration to leaves.[26] Other classes of flavonoids that can contribute color to plants or kernels are flavonols, chalcones, and aurones. Coe et al.[21] stated that about 20 loci have been identified that affect anthocyanin pigmentation in corn. In the aleurone tissue, anthocyanin pigments controlled by the *a1, a2, anl1, b Bn, brn, bz1, bz2, c1, c2, da, dek1, in, pl, pr, R, Ufo, vp1,* and *whp* loci. Pericarp coloration pigments can be affected by the *a1, c2, ch, Lc, orp1, orp2, P, pl* (sun red with light requirement), *R, Ufo,* and *whp* loci. Embryo pigmentation is affected by the *R-nj* allele at the *R* locus, by the *B-Peru* allele at the *B* locus, and by the *Sn* and *Ufo* loci. Against a

white endosperm background, the red and blue pigmentations produced by anthocyanins are easily detected and breeding selections containing these genes can be discarded from white endosperm breeding programs. However, in pericarps that carry certain alleles, such as *B-b, R-r* or *Lc,* and *pl* (sun red with light requirement), pigmentation occurs when exposed to sunlight. Thus, kernel coloration can occur when husk cover is not complete. Ideally, the white endosperm genotypes should be homozygous for *r-g b,* which would eliminate anthocyanin pigment production in the pericarp when exposed to light.

White cob color, which is desired with white endosperm kernels, is primarily controlled by the recessive *P-ww* (used interchangably with *p*) allele at the *P* locus.[21] The *P-ww* homozygote provides colorless (white) cobs. The *P-ww* allele also may be either browning (*P-wwb*) or nonbrowning (*P-www*) because of the presence of orthodihydroxy flavones and cause either production or absence, respectively, of the flavone glycoside, maysin, in silks.[21,38] Maysin inhibits the growth of the corn earworm, *Heliothis/Helicoverpa zea* (boddie),[39] but maysin may cause a "dingy" kernel pericarp that is undesirable for processed food products.[40]

Additionally, cobs may be white as a result of either the *c2 whp* genotype or the light gray-brown in *c2 whp B Pl* genotype. "In cobs, the formation of brick-red pigment ("phlobaphenes") requires *A1, C2* or *Whp* and either a *P-rr* or *P-wr* allele. If *a1* is recessive, the cob is light tan; if *c2 Whp,* light red; if *c2 whp* or *P-ww,* white. Purple anthocyanin pigments (conferred by *B Pl*) or browns (due to *a1, a2, bz1,* or *c2*) hide the brick red color."[21] Cob color may also be influenced by the brown-midrib factors, *bm1, bm2, bm3,* and *bm4,* which "… condition brown pigmentation that is prominent in the leaf midrib and is strongly evident along the vascular bundles of the leaf sheath, husks, and culm, and in the cob …."[21]

Inheritance of other kernel properties, such as starches, proteins, and lipids for white endosperm germplasms, are not different than for yellow endosperm germplasms. Waxy starch (*wx*), high amylose starch (*ae*), and high lysine (*o2*) conversions of white lines are available from several breeding programs. Cytoplasmic male sterility has been incorporated into many white endosperm inbred lines and its inheritance does not differ from the general case. Conversely, traits from white endosperm germplasm have been transferred to yellow endosperm lines. White endosperm line, *Ky21,* was the source of restorer genes for several male sterile cytoplasms.[41] A unique application for the control of gametophytic transmission was initiated by Dr. M. S. Zuber, USDA-ARS, at the University of Missouri. He incorporated the gametophytic transmission gene *Ga-s* that was derived from popcorn sources, into white endosperm inbred lines. White endosperm grain production could thus be implemented without concern of contamination by nearby yellow endosperm hybrids. This genetic isolation procedure, however, has not been utilized widely for commercial white maize production. Publicly released conversions of specialty white endosperm mutants are identified in a later section.

III. BREEDING WHITE ENDOSPERM CORN

Breeding of white endosperm corn began with the earliest plant breeders at the sites of the origin of corn; in Central and South America and in Mexico. Many of the primitive races of corn described by Goodman and Brown[42] had white endosperm and white cobs. Goodman[43] estimated that as many as 300 races of corn may have been developed. Native American civilizations that followed further shaped and reshaped the plant and kernel type, adaptation, and kernel coloration. The productive corn plant became the agricultural basis for numerous civilizations throughout the Americas (Mangelsdorf[44]). In addition to its tropical or subtropical origin, the crop was grown as far north as Canada and in nearly all except the most adverse environments found in the Americas. In addition to food, the corn grain and ear became important in religious ceremonies and many color variations were fixed. Many of the selections developed by Native Americans in the Southwest had floury white kernels with accents of purple or red. In addition to flour corns, flint, sweet, and pop types were also developed. A plethora of kernel type and coloration were available to the early European colonists first settling America.

The earliest colonists contributed as plant breeders in many significant ways. However, the most profound contribution was the fixation of the dent type by combination of a northern flint corn with a southern flour corn. This significant event has been described[42,44] and will not be discussed further except to note that one of the assumed progenitors, the southern flour corn gourdseed, has both white and yellow color kernel variations. Speculations about the flint progenitor have also been made, but it is likely that many flint-flour combinations were made and probably produced numerous color variations that could have given rise to both yellow and white endosperm types.

Mass selection, retaining desirable ears as seed for next year's crop, was practiced by most farmers prior to the 1930s.[2] Those farmers with a good eye and an instinct to select productive plant types profited from their abilities and often became suppliers of seed for neighbors. Corn selection became an occupation, or at least a sideline for extra income, for many early U.S. farmers. Although mass selection without control of male parentage was probably most common, some farmers refined their technique to include pollen control by detasseling undesirable plants. This was often done in isolation from other fields, although the value of isolation was not always appreciated. The breeding efforts of a Tennessee farmer to use pollen control in developing prolific corn was first described by H. S. Bidwell in the *American Agriculturist* of December 1867 and repeated by Jenkins.[2] White corn varieties undoubtedly benefitted frequently from such selections because white endosperm corn was preferred in the southern states and because many white open-pollinated varieties contain the word "prolific" in their name. By the late 1890s hundreds of open-pollinated varieties resulting from the efforts of either farmers or specialized corn breeders were available for use. Sturtevant[1] classified more than 500 open-pollinated varieties that he observed in the U.S. Department of Agriculture collection, from State Agricultural Experiment Station collections, and from private seedsmen. About 49% of the populations had white endosperm. He also classified the populations as dent, flint, floury, pop, and sweet. White endosperm types were found in each category and most of the sugary types were white. White endosperm varieties studied by Sturtevant[1] as well as by others are listed in Table 8.2. Jenkins[2] listed 183 white open-pollinated varieties that were recommended by State Agricultural Experiment Stations for use by farmers in their state. The years before corn breeding became based on the science of genetics were active years for corn improvement even though the procedures used were elementary. Many important traits were fixed in specific open-pollinated varieties and have served as the source of much modern corn germplasm. Unfortunately, many of the open-pollinated varieties listed in Table 8.2 are no longer available in any collection. Those with a PI number (shown in Table 8.3) are in long-term storage at the USDA's National Seed Storage Laboratory in Fort Collins, CO, and are maintained in the USDA Regional Plant Introduction Center at Ames, IA. Others may be available in either private or public collections. Useful variants may still be found within these highly variable germplasm sources, although most modern white corn breeding programs no longer utilize the early landrace varieties.

Hybrid corn became a reality in the 1930s. Jenkins[2] described the development of the new hybrid breeding procedures in great detail. Inbreeding studies begun in 1905 by Shull and East established the basic breeding procedures and value of hybrids. Shull[45] published suggetions about the inbred-hybrid breeding method in 1908 and 1909. The implications and suggestions for the utilization of corn hybrids were not realized, however, until Jones[46] proposed an economical and practical improvement of seed production with the use of double-cross hybrids. Hybrid corn breeding started in earnest shortly after 1920 when F. D. Richey of the USDA, organized federal and State Agricultural Experiment Station programs into a national program.[2] White and yellow inbred lines were produced from many open-pollinated varieties by the same procedure of repeated selfing followed by visual selection. The earliest released hybrids were yellow endosperm hybrids adapted to the central or northern U.S. Corn Belt and depended on the advantageous hybrid vigor obtained from combination of diverse inbred sources, i.e., Lancaster Sure Crop derived inbreds crossed with Reid Yellow Dent derived inbreds. Hybrids for the southern states and white hybrids

TABLE 8.2
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
		Open-pollinated varieties, dent and presumed dent		
90-day white	MO, IL	W	Farmer selection	269747
Adams champion white pearl	LA	W	Farmer selection	
Adams early[a]	NY	W	Farmer selection	
Adams extra early[a]	NY	W	Farmer selection	
Allegheny	NC	W	Farmer selection	76023
Anderson's white	NC	W	Farmer selection	76039
Armstrong white[a]	NE	W	Farmer selection	
Ayer's prolific	NC	W	Farmer selection	75989
Bakersville	NC	W	Farmer selection	82760
Baldwin branching[a]	MA	W	Farmer selection	
Benton white[a]	CN	W	Farmer selection	
Bessarabia[a]	MA	W	Farmer selection	
Best's prolific	KY	W	Farmer selection	
Big seed[a]	TX	W	Farmer selection	
Big. St. Charles	MO	W	Farmer selection	269752
Big white	KY, TN	W	Farmer selection	
Bigg's two ear	NC	W	Farmer selection	82744
Blattel white	MO	W	Farmer selection	221883
Blount prolific[a]	MO, PA, MA	W	Farmer selection	
Blue speckled	KY	W	Farmer selection	
Blue tick	NC	W	Farmer selection	76053
Boone county white[a]	IN, KY, MO	W	Farmer selection	221866 233008
Bowman's cole creek	KS	W	Farmer selection	222635
Boyd's special	NC	W	Farmer selection	76030
Brady white	NE	W	Farmer selection	
Bravo padella white	TX	W	Farmer selection	401762
Breve padilla white	TX	W	Farmer selection	414178
Bulk 7	ND	W	Collection	269755
Burke's garden white	VA	W	Farmer selection	311248
Burr white[a]	IL	W	Farmer selection	
Burrill and Whitman ensilage[a]	IL	W	Farmer selection	
Butler Co. white	KS	W	Farmer selection	222640
Calhoun red cob[a]	LA	W	Farmer selection	
Calico[a]	IL	W	Farmer selection	
Caragua[a]	NY	W	Farmer selection	
Carolina shoe peg[a]	NY	W	Farmer selection	
Carr corn[a]	NC	W	Farmer selection	
Cary[a]	NY	W	Farmer selection	
Cash white	OH	W	Farmer selection	278711
Cass County white	NE	W	Farmer selection	
Cassel white	KS, IA	W	Farmer selection	222620 214287
Challender blue and white	KS	W	Collection	222629
Champion early white pearl[a]	IL	W	Farmer selection	
Champion of the north	IL	W	Farmer selection	
Champion white dent[a]	OH	W	Farmer selection	
Champion white pearl[a]	IL, KS	W	Farmer selection	

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Cherokee[a]	NY	W	Farmer selection	
China corn[a]	CN	W	Farmer selection	
Chisholm redcob	TX	W	Farmer selection	
Clark premium 110 day	IL	W	Farmer selection	
Clayton bread[a]	TX	W	Farmer selection	
Cocke prolific[a]	NC, TN, KY	W	Farmer selection	82753
Colorado white[a]	CO	W	Farmer selection	
Commercial white	KS, MO	W	Farmer selection	222643 269746
Common early white[a]	IL	W	Farmer selection	
Common[a]	TN, OH	W	Farmer selection	
Cottrell's best white	IA	W	Farmer selection	214288
Cranberry[a]	IL	W	Farmer selection	
Crawford Co. white	KS	W	Farmer selection	222626
Crouch's white dent	MO	W	Farmer selection	401760
Currituck[a]	NC	W	Farmer selection	
Dakota white	ND, SD	W	Farmer selection	219875 213784
Davenport dent[a]	MI	W	Farmer selection	
Davenport roper	NC	WY	Farmer selection	82746
Delano improved[a]	NE	W	Farmer selection	
Delta prolific white	AK	W	Farmer selection	221871
Dill white dent	KS	W	Farmer selection	221895
Douthet's white	VA	W	Farmer selection	311239
Dungan white prolific[a]	OH	W	Farmer selection	
Early Adams[a]	NY	W	Farmer selection	
Early giant white dent[a]	OH	W	Farmer selection	
Early white	IA, NY	W	Farmer selection	214289
Early white dawn[a]	OH	W	Farmer selection	
Early white pearl[a]	MO	W	Farmer selection	
Early white prolific[a]	PA	W	Farmer selection	
Early Wisconsin white cap[a]	IL	W	Farmer selection	
Ellis	NC	WY	Farmer selection	76011
Emges white	IA	W	Farmer selection	214290
Etherington (mountain corn)	KY	W	Farmer selection	
Eureka	VA, KY	W	Farmer selection	311230
Eureka ensilage	NC	W	Farmer selection	82748
Extra early Adams[a]	NY	W	Farmer selection	
Farmers pride[a]	AL	W	Farmer selection	
Ferrell	NC	W	Farmer selection	76016
First premium[a]	TX	W	Farmer selection	
Fitsgerald, R.L.	NC	W	Farmer selection	75997
Forident white	FL	W	Collection 78% Whately prolific	
Four County white	IA	W	Collection	
Forsyth favorite[a]	OH	W	Farmer selection	
Fosters white	OH	W	Farmer selection	278714
Freed white	KS	W	Farmer selection	222619
Furgeson's	NC	W	Farmer selection	76028

continued

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Giant broad grain[a]	AL, TX	W	Farmer selection	
Giant Normandy[a]	IL	W	Farmer selection	
Gibbs special	VA	W	Farmer selection	311247
Goodman's	NC	W	Farmer selection	82750
Gould Hill prolific[a]	IL	W	Farmer selection	
Gourdseed[a]	IA, IL, TX	W	Collection	217405
				414183
				414179
Hastings prolific	AL	W	Farmer selection	
Haunschild white Golden City	MO	W	Farmer selection	233011
Hawkins improved[a]	TX	W	Farmer selection	
Haywood County	NC	W	Farmer selection	76009
Heel tap	NC	WY	Farmer selection	76022
Helm improved[a]	IL	W	Farmer selection	
Hendron bread corn[a]	LA	W	Farmer selection	
Herring	NC	W	Farmer selection	75992
Hess white[a]	OH	W	Farmer selection	
Hickory king[a]	VA, KY, TN, LA	W	Farmer selection	311237
Highland horsetooth	NC	W	Farmer selection	76004
Highsmith	NC	W	Farmer selection	75999
Hillsboro white dent	TX	W	Farmer selection	
Hiwasse mammoth[a]	IL	W	Farmer selection	
Hoilman	NC	WY	Farmer selection	82752
Holcombe prolific	NC	W	Farmer selection	76008
Hollidays	NC	W	Farmer selection	76031
Hominy[a]	IL	W	Farmer selection	
Honbarriers ensilage	NC	WY	Farmer selection	82747
Horse-tooth dent[a]	CN	W	Farmer selection	
Huffman[a]	TN, KY	W	Farmer selection	
Hughs choice[a]	IL	W	Farmer selection	
Huntley	NC	WY	Farmer selection	76014
Illinois silver mine	IL	W	Farmer selection	
Illinois white[a]	IL	W	Farmer selection	
Improved blount prolific[a]	IL	W	Farmer selection	
Iowa ideal	IA	W	Farmer selection	278723
Iowa king[a]	IL	W	Farmer selection	
Iowa silvermine	MO, KY	W	Farmer selection	221868
Israel[a]	NC	W	Farmer selection	
Jellicorse	TN	W	Farmer selection	
John Fequette	NC	W	Farmer selection	76047
Johnson	NC	W	Farmer selection	75991
Johnson County white	IA, MO, KY	W	Farmer selection	278724
				221869
Johnson's prolific	NC	W	Farmer selection	76032
Kansas king[a]	KS, LA	W	Farmer selection	
Kansas prolific[a]	KS	W	Farmer selection	
Knapp white	MO	W	Farmer selection	269745
Knowles	NC	W	Farmer selection	96931
Kyle no. 1[a]	MS	W	Farmer selection	

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Kyle no. 2[a]	MS	W	Farmer selection	
Kyle no. 4[a]	MS	W	Farmer selection	
Large white[a]	IA	W	Farmer selection	
Latham's double	NC	W	Farmer selection	75981
Lemon	NC	W	Farmer selection	82743
Limbercob	NC	W	Farmer selection	76033
Little red cob[a]	KS	W	Farmer selection	
Locklear	NC	WY	Farmer selection	76052
Long Island dent[a]	CN	W	Farmer selection	
Long Island white[a]	NY	W	Farmer selection	
Long John[a]	USDA	W	Farmer selection	
Long prairie	NC	WY	Farmer selection	82761
Long Tom[a]	NC	W	Farmer selection	
Long white dent[a]	NY	W	Farmer selection	
Mama Kat corn	NC	W	Farmer selection	76057
Mammoth ivory dent[a]	KS	W	Farmer selection	
Mammoth white dent[a]	OH	W	Farmer selection	
Mammoth white pearl	MO	W	Farmer selection	314844
Mammoth white prize[a]	NE	W	Farmer selection	
Mammoth white surprize[a]	MO, LA, IL, TX	W	Farmer selection	
Marion	NC	W	Farmer selection	96923
Maryland gourd seed[a]	IL	W	Farmer selection	
Maryland prolific[a]	LA	W	Farmer selection	
Maryland white[a]	MD	W	Farmer selection	
Mason's nant. mult. ear	NC	W	Farmer selection	76056
Mason's nantahala	NC	W	Farmer selection	82763
McCormick white	MO	W	Farmer selection	273474
McElroy white dent	TX	W	Farmer selection	
McInich white dent[a]	NE	W	Farmer selection	
McNeill's little cob	VA	W	Farmer selection	311242
Mexican june	TX	W	Collection (Mexico origin)	
Mexican june[a]	VA, TX, LA	WB	Farmer selection	311243
Middleton	NC	WY	Farmer selection	76017
Mill County white[a]	IA	W	Farmer selection	
Miller's special	NC	WY	Farmer selection	76025
Montgomery Co. blue and white	KS	W	Farmer selection	222624
Montgomery Co. white	KS	W	Farmer selection	222623
Montgomery Co. white	KS	W	Farmer selection	222623
Moore white[a]	TX	W	Farmer selection	
Mosby early field[a]	TX	W	Farmer selection	
Mosby early[a]	TX, LA	W	Farmer selection	
Mosby prolific[a]	KS, LA	W	Farmer selection	
Mosby[a]	MS	W	Farmer selection	
Mosby-Graham	AL	W	Farmer selection	
Moss, R.R.	NC	WY	Farmer selection	82751
Muse[a]	MS	W	Farmer selection	
N.B. Johnson	NC	WY	Farmer selection	96930
Nagel white dent	SD	W	Farmer selection	414186

continued

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Native Texas dent corn	TX	W	Farmer selection	414182
Neal's paymaster	AK, TN, AL	W	Farmer selection	221872
Nebraska white prize[a]	NE	W	Farmer selection	
Neosho Co. white	KS	W	Farmer selection	222638
New madrid white[a]	LA	W	Farmer selection	
Newsome	NC	W	Farmer selection	75996
Nixon	NC	W	Farmer selection	82755
No cob	NC	WY	Farmer selection	96922
Normandy giant[a]	KS	W	Farmer selection	
Ober, Paul	NC	W	Farmer selection	96921
Ohio white cap[a]	IL	W	Farmer selection	
Oklahoma silvermine	VA	W	Farmer selection	311234
Old cabin home[a]	IL	W	Farmer selection	
Osborn white dent	MO	W	Farmer selection	221896
Ozark white	MO	W	Farmer selection	269748
P53	AR	W	Collection	218182
P54	AR	W	Collection	218183
P69	AR	W	Collection	218189
Parrish[a]	NY	W	Farmer selection	
Pate	NC	W	Farmer selection	76024
Patterson[a]	LA	W	Farmer selection	
Peabody[a]	MO	W	Farmer selection	
Piasa king[a]	KS	W	Farmer selection	
Piasa pet[a]	IL	W	Farmer selection	
Pioneer	ND	W	Farmer selection	219887
Poor boy	NC	W	Farmer selection	82759
Powell's large grain	NC	W	Farmer selection	96928
Predominantly white	AR	W	Havasupai Indians	317679
Premium white dent[a]	OH	W	Farmer selection	
Premium white[a]	IL	W	Farmer selection	
Priceton[a]	IL	W	Farmer selection	
Pride of saline	IA, KS, KY	W	Farmer selection	214295 222639
Pride of the South[a]	KS	W	Farmer selection	
Proctor bread[a]	MO	W	Farmer selection	
Prolific[a]	TN	W	Farmer selection	
Quitman's white dent	MO	W	Farmer selection	269741
R.D. Howard	NC	W	Farmer selection	75983
Ragan white[a]	MO	W	Farmer selection	
Red cob ensilage[a]	MO	W	Farmer selection	
Red cob Garrett	KY	W	Farmer selection	
Renfro[a]	AL	W	Farmer selection	
Richards white	KS	W	Farmer selection	222617
Rio Padilla	TX	W	Farmer selection	414180
Robertson white dent[a]	TN	W	Farmer selection	
Rockdale	TN	W	Farmer selection	
Rural heavy dent[a]	IL	W	Farmer selection	
Rustler[a]	ND, TX	W	Farmer selection	219891
Rustlers white dent	MO	W	Farmer selection	269742

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Salzer ensilage[a]	CN	W	Farmer selection	
Seven Springs Taylor	NC	W	Farmer selection	82756
Shannon no. 1 and no. 2	LA	W	Farmer selection	
Shannon no. 3[a]	LA	W	Farmer selection	
Shawnee white	KS	W	Farmer selection	222634
Shoe peg[a]	MO, NC, KY, NY	W	Farmer selection	269743
				76046
Short stalk prolific	KY	W	Farmer selection	
Sibley mammoth[a]	NY	W	Farmer selection	
Silver king	WI	W	Farmer selection	280853
Silvermine	TX	W	Farmer selection	
Smith	NC	W	Farmer selection	76027
Smith favorite[a]	IL	W	Farmer selection	
Smith improved striped[a]	IL	W	Farmer selection	
Smith improved white	IL	W	Farmer selection	
Smith mixed dent[a]	IL	WYR	Farmer selection	
Smith premium white dent[a]	IL	W	Farmer selection	
Smith white[a]	NE	W	Farmer selection	
Smith's shoe peg	NC	WY	Farmer selection	76049
Smithwick two ear	NC	W	Farmer selection	96932
Southern beauty	NC	W	Farmer selection	82745
St. Charles white[a]	KS, MO, OH, TX	W	Farmer selection	222627
				221879
				269753
Stowe bread[a]	LA	W	Farmer selection	
Strawberry[a]	NC	W	Farmer selection	
Strother white	NC	W	Farmer selection	82757
Sutton	NC	W	Farmer selection	65990
Taylor, John	NC	W	Farmer selection	82749
Tennessee red cob	NC, VA, KY	W	Farmer selection	82754
				311235
The crowder[a]	NC	W	Farmer selection	
Thomas utility white corn	IA	W	Farmer selection	214297
Thompson's celebrated prolific[a]	LA	W	Farmer selection	
Thompson's dent[a]	MO	W	Farmer selection	
Thompson's prolific[a]	KY, AL	W	Farmer selection	
Thousand fold[a]	NY	W	Farmer selection	
Upchurch strawberry[a]	NY	W	Farmer selection	
Valle Crucis	NC	WY	Farmer selection	82764
Virginia horse tooth[a]	TX	W	Farmer selection	
Virginia mammoth[a]	MO	W	Farmer selection	
Watson[a]	TN	W	Farmer selection	
Weekley improved	NC	W	Farmer selection	
Weekley's	NC	W	Farmer selection	76038
Weevil proof[a]	MS	W	Farmer selection	
Welborn conscience[a]	TX	W	Farmer selection	
Welborn pet[a]	LA	W	Farmer selection	
Welborn prolific[a]	TX	W	Farmer selection	

continued

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Whatley	AL	W	Farmer selection	
White Armstrong[a]	NE	W	Farmer selection	
White cap dent[a]	OH	W	Farmer selection	
White cap[a]	IL, NY, OH	WY	Farmer selection	
White champion[a]	WV	W	Farmer selection	
White dent[a]	MO, VA, CN	W	Farmer selection	363067
				233001
				233012
				221885
				311232
White giant Normandy[a]	AL, MO	W	Farmer selection	
White hunt[a]	IL	W	Farmer selection	
White june	TX	W	Farmer selection	
White mammoth[a]	KS	W	Farmer selection	
White Maryland gourdseed	LA	W	Farmer selection	
White pearl[a]	IL	W	Farmer selection	
White prolific[a]	OH	W	Farmer selection	
White queen[a]	IL	W	Farmer selection	
White rockdale[a]	LA	W	Farmer selection	
White shoe peg[a]	LA	W	Farmer selection	
White St. Charles[a]	LA	W	Farmer selection	
White surecropper	AK, TX	W	Farmer selection	221873
White synthetic 1	KY	W	Farmer selection	
White Thomas	TX	W	Farmer selection	
White tuxpan	TX	W	Farmer selection	
White Wabash[a]	IL	W	Farmer selection	
Whitemaster hybrid	TX	W	Farmer selection	401763
Whitney corn[a]	MI	W	Farmer selection	
Wilkes County	NC	W	Farmer selection	75984
Windus white dent 1918	CO	W	Farmer selection	217481
Winner	KY	W	Farmer selection	
Wisconsin earliest dent[a]	OH	W	Farmer selection	
Wisconsin white dent[a]	NY	W	Farmer selection	
Wood's dixie	VA	W	Farmer selection	311241
Wood's prolific	NC	W	Farmer selection	75982
Woodworth eighty day[a]	IL	W	Farmer selection	
Wright	NC	W	Farmer selection	76029
Zeigler ninety day[a]	IL	W	Farmer selection	
Open-pollinated varieties, flint				
Bulk 1	ND	W	Collection	222305
Connecticut white[a]	NY	W	Farmer selection	
Dakota white flint	ND	W	Farmer selection	213790
Eight-rowed red	CN	W	Farmer selection	
Eight-rowed red glazed	NY	W	Farmer selection	
Eight-rowed white[a]	NY	W	Farmer selection	
Forty day early[a]	NY	W	Farmer selection	
Gentry early market[a]	TX	W	Farmer selection	
Hominy[a]	IL, NY	W	Farmer selection	

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Jimmy T.[a]	RI	W	Farmer selection	
Lackawaxen[a]	TX	W	Farmer selection	
Large white flint[a]	LA	W	Farmer selection	
Large white[a]	PA	W	Farmer selection	
Long white flint[a]	TX	W	Farmer selection	
Longfellow flint	KY	W	Farmer selection	
Mandan flint[a]	MI	WYBS	Farmer selection	
Mandan white flint	ND, KY	W	Farmer selection	213802
Red blade field corn[a]	CN	W	Farmer selection	
Red blaze[a]	CN	W	Farmer selection	
Red noze white[a]	CN	W	Farmer selection	
Rhode island cap[a]	RI	W	Farmer selection	
Rhode Island double white cap	ME	W	Farmer selection	414175
Rhode Island premium[a]	CN	W	Farmer selection	
Rhode Island white cap[a]	CN, NY	W	Farmer selection	
Row 14, 1918 white Flint	CO	W	Farmer selection	217491
Rural thoroughbred[a]	NY	W	Farmer selection	
Sanford early[a]	MA	W	Farmer selection	
Sanford[a]	NY	W	Farmer selection	
Seneca hominy corn	NE	WB	Farmer selection	401754
Silver white[a]	IL	W	Farmer selection	
Smutty white[a]	NY	W	Farmer selection	
Spanish	IA	W	Collection	311249
Squaw[a]	MN	W	Farmer selection	
Tarahumari Indian[a]	NY	W	Collection	
Twelve-rowed white[a]	NY	W	Farmer selection	
White Australian[a]	MI	W	Farmer selection	
White Flint[a]	MO, NY	W	Farmer selection	
White pearl[a]	GA, NY	W	Farmer selection	
White smut nose[a]	MI	W	Farmer selection	
Wisconsin white flint[a]	MO	W	Farmer selection	
Open-pollinated varieties, floury and presumed floury				
All white supai corn	AR	W	Havasupai Indians	317676
Apache red	IA	WY	Apache tribe	213731
Apache red cob	IA	WB	Apache tribe	213729
Apache white	IA	W	Apache tribe	213728
Arapaho white	IA	W	Kiowa tribe	213753
Brazilian flour[a]	KS, LA	W		
Bulk 4	ND	W	Collection	222308
Cuzco[a]	NY	W	Collection	
Four corn[a]	WY	W	Farmer selection	
Mandan (squaw)[a]	NY	WYBP	Farmer selection	
P1	NM	W	Collection	218130
P2	NM	W	Collection	218131
P3	NM	W	Collection	218132
P34	AR	W	Collection	218163
P3D	NM	W	Collection	218133

continued

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
P40	NM	WYB	Collection	218169
P47	AR	W	Collection	218176
P5	NM	W	Collection	218135
P59	AR	W	Collection	218187
P7	NM	W	Collection	218137
P8	NM	W	Collection	218238
P9	NM	W	Collection	218239
Red River[a]	NY	W	Farmer selection	
Sand Padro Indian[a]	NY	WB	Farmer selection	
Seeds white and blue	IA	WB	Cheyenne tribe	213746
Seeds white and blue	IA	WB	Cherokee tribe	213743
Seeds white and blue	IA	WB	Five tribes	213750
Seeds white and blue	IA	WB	Arikara tribe	213742
Seeds white and purple	IA	WP	Shawnee tribe	213758
Seeds white and purple	IA	WP	Sac and Fox tribe	213768
Seeds white and yellow	IA	WY	Shoshoni tribe	213769
Seeds white and yellow	IA	WY	Blackfeet tribe	213760
Seeds white, yellow, and blue	IA	WYB	Navajo tribe	213738
Sioux tribe	NE	W	Sioux tribe	401755
Tuscarora[a]	NY, MO	W	Farmer selection	
White flour	SD	W	Farmer selection	317681
Wyandotte[a]	MO	W	Farmer selection	
Zuni purple spotted[a]	NY	WP	Collection	
Zuni white[a]	NY	W	Collection	
Open-pollinated varieties, popcorn and presumed popcorn				
Argentine pop	IL	W	Collection	414177
Bear foot[a]	NY	W	Farmer selection	
Bulk 10	ND	W	Collection	269758
Chicago white parching[a]	NY	W	Farmer selection	
Common twelve rowed[a]	NY	W	Farmer selection	
Common white 8[a]	PA	W	Farmer selection	
Egyptian joint[a]	NY	W	Farmer selection	
Egyptian[a]	MA	W	Farmer selection	
Large eight rowed[a]	PA, MA	W	Farmer selection	
Mapledale prolific[a]	IL	W	Farmer selection	
Mapledale[a]	PA	W	Farmer selection	
Miniature[a]	NY	W	Farmer selection	
Monarch rice[a]	IL	W	Farmer selection	
New England[a]	NY	W	Farmer selection	
Nonpareil[a]	NY	W	Farmer selection	
Page striped rice[a]	IA	W	Farmer selection	
Pearl[a]	NY	W	Farmer selection	
Rice parching[a]	NY	W	Farmer selection	
Rice pop[a]	NY	W	Farmer selection	
Silver laced[a]	NY	W	Farmer selection	
Small pearl[a]	NY	W	Farmer selection	
Snow ball[a]	PA	W	Farmer selection	
WH-PAH-2	IA	W	Collection	340871

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
White hulless I	IA	W	Farmer selection	311251
White hulless II	IA	W	Farmer selection	311252
White hulless III	IA	W	Farmer selection	211253
White rice	IA	W	Farmer selection	311250
White variegated[a]	NY	WP	Farmer selection	
White's white rice[a]	IA	W	Farmer selection	
Wisconsin prolific[a]	PA	W	Farmer selection	
Open-pollinated varieties, sweet corn and presumed sweet corn				
Albany sugar[a]	IL	W	Farmer selection	
Amber cream[a]	NY	WR	Farmer selection	
Asylum sugar[a]	IL	W	Farmer selection	
Asylum[a]	NY	W	Farmer selection	
Ballard extra early[a]	IL	W	Farmer selection	
Banana sugar[a]	NY	W	Farmer selection	
Bonanza[a]	MO	W	Farmer selection	
Breck premier[a]	IL	W	Farmer selection	
Burbank early Maine[a]	IL	W	Farmer selection	
Burbank early[a]	IL	W	Farmer selection	
Burr improved[a]	NY	W	Farmer selection	
Chicago market[a]	IL	W	Farmer selection	
Clark early nonesuch[a]	PA	W	Farmer selection	
Clark nonesuch[a]	MA	W	Farmer selection	
Clark Old Colony[a]	NY	W	Farmer selection	
Cory[a]	IL	W	Farmer selection	
Country gentleman[a]	MO	W	Farmer selection	
Crosby early[a]	IL, NY	W	Farmer selection	
Crosby[a]	IL, NY	W	Farmer selection	
Darling early[a]	NY	W	Farmer selection	
Dolly dutton[a]	NY	W	Farmer selection	
Durkee[a]	IL	W	Farmer selection	
Dwarf early[a]	IL	W	Farmer selection	
Early bonanza[a]	PA	W	Farmer selection	
Early Boston market[a]	IL	W	Farmer selection	
Early Boynton[a]	IL	W	Farmer selection	
Early conqueror[a]	PA	W	Farmer selection	
Early cory[a]	NY	W	Farmer selection	
Early Des Moines[a]	IA	W	Farmer selection	
Early eight rowed[a]	NY	W	Farmer selection	
Early Genesee[a]	NY	W	Farmer selection	
Early Harrison[a]	NY	W	Farmer selection	219876
Early june	ND	W	Farmer selection	
Early mammoth[a]	PA, IL, NY	W	Farmer selection	
Early Minnesota[a]	IL	W	Farmer selection	
Early red cob[a]	IL	W	Farmer selection	
Early Rockford market[a]	IL	W	Farmer selection	
Early southern[a]	IL	W	Farmer selection	
Early sweet or sugar[a]	IL	W	Farmer selection	

continued

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Egyptian[a]	NY	W	Farmer selection	
Eight rowed[a]	IL	W	Farmer selection	
Eight rowed early[a]	NY	W	Farmer selection	
Evergreen fodder[a]	MA	W	Farmer selection	
Excelsior[a]	IL	W	Farmer selection	
Extra early Tom Thumb[a]	NY, MO	W	Farmer selection	
First crop sugar[a]	MA	W	Farmer selection	
Ford early[a]	MA	W	Farmer selection	
Harris sweet[a]		W	Farmer selection	
Henderson[a]	IL	W	Farmer selection	
Hickox hybrid[a]	NY	W	Farmer selection	
Hickox improved[a]	CN, MO, NY	W	Farmer selection	
Hickox[a]	NY	W	Farmer selection	
Honey june	TX	W	Farmer selection	
Honey[a]	IL	W	Farmer selection	
La Crosse[a]	IL	W	Farmer selection	
Landreth sugar[a]	IL	W	Farmer selection	
Large eight rowed[a]	NY, IL	W	Farmer selection	
Large excelsior[a]	NY	W	Farmer selection	
Late mammoth[a]	NY	W	Farmer selection	
Little gem[a]	IL	W	Farmer selection	
Livingston evergreen[a]	IL	W	Farmer selection	
Mammoth early[a]	PA	W	Farmer selection	
Mammoth late[a]	MO	W	Farmer selection	
Mammoth sugar[a]	MO	W	Farmer selection	
Mammoth[a]	IL, KY, NY, MA	W	Farmer selection	
Marblehead mammoth[a]	NY	W	Farmer selection	
Minnesota[a]	NY, MO	W	Farmer selection	
Moore Concord[a]	MA, NY	W	Farmer selection	
Moore early Concord[a]	NY	W	Farmer selection	
Moore early[a]	NY	W	Farmer selection	
NE plus ultra[a]	MA	W	Farmer selection	
New triumph[a]	MI	W	Farmer selection	
Nonesuch[a]	NY	W	Farmer selection	
Northern pedigree[a]	IL	W	Farmer selection	
Old Colony[a]	NY	W	Farmer selection	
Original Crosby[a]	IL	W	Farmer selection	
Pee and Kay[a]	IL	W	Farmer selection	
Perry hybrid[a]	NY	W	Farmer selection	
Potter excelsior[a]	NY, IL, MO	W	Farmer selection	
Pratt early[a]	NY	W	Farmer selection	
Red cob evergreen[a]	MO	W	Farmer selection	
Red cob[a]	IL	W	Farmer selection	
Rochester[a]	NY	W	Farmer selection	
Roslyn hybrid[a]	IL	W	Farmer selection	
Shaker early[a]	IL	W	Farmer selection	
Sonyea intermediate[a]	IL	W	Farmer selection	
Squantum sugar[a]	MO	W	Farmer selection	
Squantum[a]	NY	W	Farmer selection	

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
Stabler early[a]	NY, IL, MO	W	Farmer selection	
Stabler extra early[a]	IL	W	Farmer selection	
Stowel's evergreen[a]	ND, NY	W	Farmer selection	219893
Texas honey june	TX	W	Farmer selection	414181
Tom Thumb[a]	NY	W	Farmer selection	
Triumph[a]	NY, USDA	W	Farmer selection	
Western queen[a]	IL	W	Farmer selection	
White cory[a]	NY	W	Farmer selection	
Wyoming[a]	NY	W	Farmer selection	
Zigzag[a]	NY	W	Farmer selection	
Composite varieties, dent or presumed dent				
Blue and white composite 4	KS	W	Collection	222646
BSP1C1	IA	W	Op of hybrids	
Coroico composite	IA	W	Collection	408699
Early white composite 2	KS	W	Collection	222611
Ill Wh Syn	IL	W	BCW + IA smine + S KG + CH WH pearl	
Late white composite 6	KS	W	Collection	222612
SC Comp Syn	SC	WY	Mix 150 farmer var's	
Southern white selection	PA	W	700 white dent op's	
Texas section	PA	W	White dent hybrids	
TSGP	TN	WY	28 Var - Southern germplasm	
White 8-line Syn	TN	W	Comp/Syn of 8 white inbreds	
Synthetic varieties, dent or presumed dent				
FAWCC	GA	WY	Cimmyt and Brazil races	
FAWCC	GA	WY	Southern Sx's × Antigua	
FC	SC	W	White Syn So. GA	
GT-CEW-RS8	GA	WY	SC's of So. inbreds	
GT-CEW-UP (C2)	GA	WY	SC's of So. inbreds	
GT-SSRS-PX	GA	WY	C103 × Pop's and collections	
GT-SSRS-SX	GA	W	7 SC's chain crossed	
H Syn 73W	IN	W	FR805, H105W, + white BS17	
H Syn Lancaster/43 White	IN	W	Lanc/43 inbreds × 24 white sources	
H Syn SSS white	IN	W	B14 white recoveries	
H Syn white D	IN	W	26 white lines	
Jellicorse mass sel. (prolific)	TN	W	Jellicorse C5	
Jellicorse mass sel. (YLD)	TN	W	Jellicorse C14	
KyWs1	KY	W	(A632 × KY201)A632	531513
KyWS2	KY	W	(B73 × Ky201)Ky201	531514
KyWS3	KY	W	(Mo17 × Ky201)Mo17	531515
KyWS4	KY	W	(N28 × Ky201)N28	531516
KyWS5	KY	W	(H84 × Ky201)H84	531517
KyWS6	KY	W	(Oh545 × Ky201)Oh545	531518
KyWVS	KY	W	Sel's from exotic	536518
Mol17 white composite	MO	W	15 white Mo17 Sel's	
MoSQA cycle 4	MO	W	9 white lines	
RFC-RM1	GA	WY	Caribbean coll. × corn belt	

continued

TABLE 8.2 (CONTINUED)
Open-Pollinated White Endosperm Varieties Developed in Different Areas of the U.S.[a]

Name	State[b]	Kernel color[c]	Pedigree	PI no.[d]
SA white	PA	W	Vir res sel SAF Syn's	
SC40W	SC	W	F44 lines × CX2346W	
South African white	TN	W	Syn from Natal S. Afr	
South Dakota white corn Syn	SD	W	Farmer selection	
TELE-13	TN	WY	12 Early low ear lines -C13	
TLLE-13	TN	WY	8 Late low ear lines -C13	
TSR-C4	TN	WY	12 INB Syn smut -C4	
TVRDW-C5	TN	WY	16 Inb Syn vir - C5	
Population crosses, dent				
Lancaster × Mexican Syn	KY	WY	Lancaster × Mexican synthetic	
NCIaDDC × COAH 8	KY	WY	(Ia4570 × Ia4810) × Tuxpeno	
NCIaDDC × T2	KY	WY	(Ia4570 × Ia4810) × Tuxpeno	
NCIaDDC × TAMPS 3	KY	WY	(Ia4570 × Ia4810) × Tuxpeno	
VS2W	VA	W	Lehna × MN Yel Syn ASD	
Synthetic varieties, dent, oil, or protein selections				
Illinois high oil	IL	W	Sel from Burr white	
Illinois high protein	IL	W	Sel from Burr white	
Illinois low oil	IL	W	Sel from Burr white	
Illinois low protein	IL	W	Sel from Burr white	
Synthetic varieties, sweet corn				
Swt CD Sel. RM	GA	WY	Sweet corn Sx's	

[a] As noted by Sturtevant[1]; may have been reported by others sources as well. Other names from Henderson;[61–70] reports of the North Central Corn Breeders Research Conference, the North Eastern Corn Improvement Conference, and the Southern Corn Improvement Conference; the National Germplasm Resources listing; numerous texts; and personal communications.
[b] State of origin or origin of collection.
[c] W = white; WY = mixed white and yellow; WB = mixed white and blue; WYR = mixed, white, yellow, and red; etc.
[d] As provided by the North Central Regional Plant Introduction Station, Ames, IA.

appeared soon thereafter but differed slightly in composition. The first released white hybrid was developed by Dr. Kinney at the Kentucky Agricultural Experiment Station and used inbreds developed from a single open-pollinated variety, Boone County White.[47,48] Similar results were obtained at the Tennessee Agricultural Experiment Station using the Neal Paymaster variety. Comparable results were not obtained with all open-pollinated varieties of that era and attest to the broad genetic base that had been included in some varieties.[47] In most respects, the breeding of white and yellow endosperm corn hybrids followed the same procedures, especially when the source of inbreds was existing open pollinated varieties.

Selfing with selection (pedigree selection), evaluation of inbreds in single crosses and later evaluation of inbreds in topcrosses, and prediction of double-cross hybrids from single-cross data were effective procedures for use with any source population, yellow, white, or otherwise. Reviews of types and effectiveness of corn breeding procedures are available from several sources.[44,47,49–53] The uniqueness and progress of white endosperm corn breeding may be best evaluated from examination of the inbred releases over time. Table 8.3 lists most of the known white inbreds, classified by state of origin, and includes information about date of release, genetic source or pedigree, and an indication of the breeding procedure used in the inbred's development. The years

TABLE 8.3
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
Arkansas			
AR101	Ark SWCB Syn	SYN	1976
AR102	Ark SWCB Syn	SYN	1980
Florida			
F1-18	UN	UN	UN
F4-32	UN	UN	UN
F5-11	UN	UN	UN
F11-129	UN	UN	UN
Georgia			
GE129	Neal's paymaster	OP	1961
GE180	From PI175334 (Nepal)	OP	1984
GA203	T61 × NC37	2X	1980
GA209	T61 × NC37	2X	1971
GA313	Coker 811 × GA24/6	BC	1980
GE281	Whatley	OP	1961
GE311	Huffman	OP	1961
Iowa			
1445 (SW)	Country gentlemen white	OP	1952
1612 (SW)	Country gentlemen white	OP	1952
1627 (SW)	Country gentlemen white	OP	1952
4135 (SW)	Country gentlemen white	OP	1952
4137 (SW)	Country gentlemen white	OP	1952
4Co31	4 Country white	COMP	1952
4Co63	4 Country white	COMP	1952
4Co82	4 Country white	COMP	1952
4Co101	4 Country white	COMP	1952
Illinois-IFSI			
FR801W	C103 × CI61	2X	1972
FR802W	C103 × CI61	2X	1972
FR803W	Oh43 × K41	2X	1972
FR804W	B37 × 33–16	2X	1972
FR805W	B37 × 33–16	2X	1976
FR806W	N28/3 × white	BC	1984
FR807W	W64A/3 × H28	BC	1984
FR808W	B73 × FR805W	2X	1984
FR809W	B73 × FR805W	2X	1984
FR810W	Mo17 × CI66	2X	1984
FR811	FRCI64 × (Oh41 × C103/2)S3	2X	1985
FR812	FRCI64 × (Oh41 × C103/2)S3	2X	1985
FR813	FRMo17/2 × FR33-16	BC	1985
FR814	(A632/3 × K6)S3 × FR805	2X	1985
FR815	(A632/3 × K6)S3 × FR805	2X	1986
FR816	FR806 × Ky216	2X	1986
FR817	FR806 × (CI66 × Ky228)	3X	1986
FR818	Ky228 × W729C	2X	1987

continued

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
FR819	B73/5 × Ky228	BC	1990
FR820	FR806 × FR805	2X	1990
FR821	FR16 × FR806	2X	1990
FR822	Ky228 × FR808	2X	1990
Illinois			
R6	Surecropper	OP	1950
R7	Neal's paymaster	OP	1950
R8	Texas surecropper	OP	1950
R9	Neal's paymaster	OP	1950
R11	Nicholson Drowth resistant	OP	1950
R13	Pride of Saline	OP	1950
R14	Pride of Saline	OP	1950
R15	Burkhardt	OP	1950
R16	Burkhardt	OP	1950
R17	Commercial white	OP	1950
R18	Hominy	OP	1950
R19	Hominy	OP	1950
R20	McLurkin	OP	1950
R21	Champion white pearl	OP	1950
R22	Champion white pearl	OP	1950
R23	Champion white pearl	OP	1950
R24	Champion white pearl	OP	1950
R25	Champion white pearl	OP	1950
R26	Champion white pearl	OP	1950
R27	Champion white pearl	OP	1950
R28	Champion white pearl	OP	1950
R29	Champion white pearl	OP	1950
R30	Champion white pearl	OP	1950
R31	Champion white pearl	OP	1952
R47	Burr white	OP	1952
R49	Harmon white dent	OP	1952
R43	ILL high oil	SYN	1952E
R44	ILL high oil	SYN	1952E
R77	IHP × 38–11/2	POPX	1960E
R85	IHP/2 × Hy	POPX	1960E
R88	IHO × L317/2	POPX	1960E
R90	IHP × 38–11/2	POPX	1960E
R119	IHP/2 × 38–11	POPX	1960E
R120	IHP/2 × 187–2	POPX	1960E
R121	IHO/2 × 38–11	POPX	1960E
R122	IHO X L317/2	POPX	1960E
R144	(WF9 × R30) WF9/3	BC	1960E
R145	(Hy2 × R30) Hy2/3	BC	1960E
R146	(M14 × R30) M14/2	BC	1960E
R147	(38–11 × H21) 38–11/3	BC	1960E
R148	(38–11 × R30) 38–11/3	BC	1960E
R149	(L317 × K64) L317/3	BC	1960E
R150	(187–2 × Ky27) 187–2/3	BC	1960E

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
RCI64Ht1(A),BC5	CI64 × Ht1(A) source	BC	1974
RKY27Jt1(A),BC5	Ky27 × Ht1(A) source	BC	1974
Indiana			
LX33	Luxes BCW	OP	1934
LX2-2	Luxes BCW	OP	1935
33–16	Johnson Co. white	OP	1938
H21	Johnson Co. white	OP	1946
H22	Johnson Co. white	OP	1946
H23	Pride of Saline	OP	1946
K6	Pride of Saline	OP	1948
H25	Johnson Co. white	OP	1953
H26	(K18 × M14)M14	BC	1953
H27	(K8 × L)L	BC	1953
H28	(K60 × Hy)Hy	BC	1953
H29	I205	OP	1953
H30	(K64 × 38–11)38–11/2	BC	1953
H31	ILL. Low oil	SYN	1953
471-V6 (SW)	Aunt Mary's sweet	OP	1956
81–1 (SW)	Honey June	OP	1956
166 (Sw)	81–1 X14N	2X	1960
33–16TMS	UN	UN	1964
K41TRf	K41	BC	1964
K61TRf	K61(K55)	BC	1964
WR4533 (POP)	White rice	OP	1964
HP303W (POP)	Hulless × S. American	POPX	1976
HP304W (POP)	UN	UN	1976
H104	H26 × A619	2X	1981
H105	33–16 × A632	2X	1981
H106	H28 × H84	2X	1981
H118	Mayorbella	OP	1985
H122	(74-R336 × FR805)B73/2	BC	1988
H124	H Syn white D	SYN	1990
Kansas			
K55	Pride of Saline	OP	1946
K64	Pride of Saline	OP	1946
K41	Pride of Saline	OP	1948
K63	Pride of Saline	OP	1948
K44	Pride of Saline	OP	1952
K6	Pride of Saline	OP	1952
K61	Pride of Saline	OP	1952
K811o2	(K55,K41, & o2)	3X	1972
K812o2	(K699 & o2)	2X	1972
K813o2	(K740 & o2)	2X	1972
K814o2	(Pride of Saline & o2)	POPX	1972
K816o2	(K41 & o2)	2X	1972
K301	(B73 × white)B73/8	BC	1980

continued

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
K302	(B68 × white)B68 × (K41 × H28)	4X	1980
K303	(Mo17 × K41)Mo17/7	BC	1980
K304	(A632 × K41)A632/8	BC	1980
K305	(Va26 × H28)Va26/7	BC	1980
K306	(H98 × K41-H28)H98/8	BC	1980
Kentucky			
Ky124	White source	UN	1937
Ky132	White source	UN	1937
Ky20	Ky17	Inbred	1937
Ky31	Ky28	Inbred	1937
Ky34	White source	UN	1937
Ky50	White source	UN	1937
Ky52	Ky49	Inbred	1937
Ky85	Ky58	Inbred	1939
Ky11B	Boone Co. white	OP	1940
Ky122	Boone Co. white	OP	1940
Ky13	Boone Co. white	OP	1940
Ky21	Boone Co. white	OP	1940
Ky27	Boone Co. white	OP	1940
Ky30A	Boone Co. white	OP	1940
Ky32	White source	UN	1940
Ky39	Boone Co. white	OP	1940
Ky49	Boone Co. white	OP	1940
Ky54	Ky51	Inbred	1940
Ky56	Boone Co. white	OP	1940
Ky58	Boone Co. white	OP	1940
Ky59	Ky56	Inbred	1940
Ky92	Ky89	Inbred	1940
KY201	White pearl	OP	1960
KY209	Ky30A × 38–11	2X	1963
Ky211	WF9 × Ky39	2X	1963
Ky211cms	WF9 × Ky39	2X	1963
CI64rf	(Ky39 × CI64)CI64/2⊗BC⊗BC/2	BC	1967
CI64Trf	(WF9Tms × CI64/4)CI64rf/2	BC	1967
Ky216	Webb-Watson 10	OP	1967
Ky217	KY white Syn 19	SYN	1967
Ky222	Iowa Syn B	SYN	1967
Ky225	NCIaDDC × Coah 8	POPX	1967
Ky226	NCIaDDC × Coah 8	POPX	1967
Ky228	Pride of Saline	OP	1967
Louisiana			
L10	UN	UN	1957
L2-2	UN	UN	1960
L44	UN	UN	1960
L62	UN	UN	1960
L90	UN	UN	1960
L91	UN	UN	1960

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
L108	White tuxpeno	OP	1972
L163	L55 × L90	2X	1972
L300	ACE 996.41 × T91	2X	1972
L329	CR811 × L101	2X	1972
L330	CR811 × L101	2X	1972
L336	F44 × 5503W	2X	1972
L337	MP313 × L2-2	2X	1972
Massachusetts			
H84 (SW)	Stowell evergreen cross	POPX	1948
MA76.32 (SW)	Unknown	UN	1984
MA76.33 (SW)	Unknown	UN	1984
MA76.34 (SW)	Unknown	UN	1984
Minnesota			
A166	49(Minn 13) × 15–28 (Rustler)	POPX	1946
A188	4-29(Sil king) × 64(NW dent)/4	POPX	1946
A171	Composite	COMP	1948
A34	Rustler	OP	1948
A305	1(Silver king) × H	2X	1952
A308	15(Rustler) × 8–29(Purdue YD)	2X	1952
A310	6(Silver king) × H(Reid YD)	2X	1952
A312	15 × 8–29	2X	1952
C15	Rustler	OP	1952
C16	Rustler	OP	1952
C19	Rustler	OP	1952
C20	Rustler	OP	1952
Imp.91	((88 × 91)91/2)Silver King	BC	1952
Missouri			
Mo1W	Mo22 × WF9/2	BC	1957
Mo21R	H22 × Mo21A/5	BC	1957
Mo22W	Laguna OP	OP	1957
Mo2RF	B2 × 33–16/3	BC	1959
K10	Pride of Saline	OP	1960
Mo11	Mo22 × WF9/2	BC	1960
Mo8W	Pipe corn OP	OP	1960
Mo9W	Pipe corn OP	OP	1960
Mo14W	Mo22 × WF9	2X	1963
Mo15W	OP Pipe	OP	1963
Mo16W	OP Pipe	OP	1963
K55NR	K55 (H28 Tms source)	Inbred	1967
K55Tms	K55 (H28 Tms source)	Inbred	1967
Mo301ae	(K55 × aeK)K55/6 sel	BC	1967
Mo302ae	(K64 × aeK)K64/5 sel	BC	1967
Mo311ae	(Mo8W × aeK)Mo8W/2 sel	BC	1967
Mo18W	(WF9 × Mo22)Mo22	BC	1968
Mo317ae	(Mo1W × aeK)MolW/5 sel	BC	1969
Mo318ae	(Mo9W × aeK)Mo9W/3 sel	BC	1969

continued

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
Mo319ae	(H28 × aeK)H28/3 sel	BC	1969
Mo320ae	(Ky122 × aeK)Ky122/5 sel	BC	1969
Mo322ae	(Mp303 × aeK)Mp303/5 sel	BC	1969
Mo323ae	(aeK × Cuzco)Cuzco/4 sel	BC	1969
Mo324ae	(Ga153 × aeK)Ga153/5 sel	BC	1969
Mo20W	N6Xmo22	2X	1971
Mo501W (Ga-s)	K55 Ga-s	BC	1972
Mo502W (Ga-s)	Ky49 Ga-s	BC	1972
Mo503W (Ga-s)	CI127 Ga-s	BC	1972
Mo504W (Ga-s)	CI64 Ga-s	BC	1972
Mo505W (Ga-s)	H25 Ga-s	BC	1972
Mo506W (Ga-s)	K6 Ga-s	BC	1972
Mo507W (Ga-s)	T115 Ga-s	BC	1972
Mo508W (Ga-s)	H30 Ga-s	BC	1972
Mo509W (Ga-s)	T111 Ga-s	BC	1972
K41o2	(K41 × o2o2)K41/5	BC	1973
K55o2	(K55 × o2o2)K55/5	BC	1973
Mo20Wo2	(Mo20W × o2o2)Mo20W/5	BC	1973
Mo2Rfo2	(Mo2RF × o2o2)Mo2RF/5	BC	1973
Mo23W	(K10 × Ky49/Mamm white pearl)	POPX	1976
Mo24W	(K10 × Ky49/Ziler Hi-Cob)	POPX	1976
Mo25W	(K10 × Ky49/Ziler Hi-Cob)	POPX	1976
Mo26W	MoSQAC4S5	SYN	1976
Mo27W	MOSQAC4S5	SYN	1976
Mo28W	MoSQAC4S5	SYN	1976
Mo29W	MoSQAC4S5	SYN	1976
Mo30W	MoSQAC4S5	SYN	1976
Mo31W	MoSQAC4S5	SYN	1976
Mo32W	MoSQAC4S5	SYN	1976
Mo33W	MoSQAC4S5	SYN	1976
Mo510W (Ga-s)	N72 Ga-s	BC	1976
Mo511W (Ga-s)	(A509 × N72/2)S9 Ga-s	BC	1976
Mo512AW (Ga-s)	W64A × K6Ga-s)S10	2X	1976
Mo512BW (Ga-s)	(W64A × K6Ga-s)S9	2X	1976
Mississippi			
MP305	Jellicorse	OP	1961
MP307	Cocke prolific	OP	1961
MP311	Whatley	OP	1961
MP339	T61 × Hill yellow dent	POPX	1971
MP313E	Tuxpan	OP	1988
North Carolina			
NC31	Bigg's two ear	OP	UN
NC32	Bigg's two ear	OP	UN
NC33	Weekleys impr OP	OP	1955
NC34 (FL)	Weekleys impr OP	OP	1955
NC35	Weekley's	UN	UN
NC36	Weekley's	UN	UN

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
NC37	Weekley's	UN	UN
NC38	Weekley's	UN	UN
NC39	Weekley's	UN	UN
NC40	Southern beauty	UN	UN
NC41	Southern beauty	UN	UN
NC42	Southern beauty	UN	UN
NC43	Southern beauty	UN	UN
NC44	Southern beauty	UN	UN
NC45	Latham's double	UN	UN
NC46	Latham's double	UN	UN
NC47	Latham's double	UN	UN
NC53	Highland horsetooth	UN	UN
NC54	Highland horsetooth	UN	UN
NC55	Highland horsetooth	UN	UN
NC56	Highland horsetooth	UN	UN
NC57	Southern beauty	UN	UN
NC58	Southern beauty	UN	UN
NC59	Weekley's	UN	UN
NC60	Weekley's	UN	UN
NC296 (FL, Ga-s)	Pioneer X105A × H-5	8X	1990
NC296A (FL, Ga-s)	NC296	Inbred	1991
North Dakota			
ND304W	(B73 × ND408) × W23yy	3X	1983
Nebraska			
N50	St. Charles white	OP	UN
N52	St. Charles white	OP	UN
N72	St. Charles white	OP	1950
N75	Nebraska white prize	OP	UN
N78		OP	UN
New York			
NY2 (POP)	Onodaga	OP	1956
NYP504 (POP)	Jap hulless	OP	1976
NYP505 (POP)	Jap hulless	OP	1976
South Carolina			
SC152	Latham's double × T61-TX61M	POPX	1966
SC235R3 (cms)	F3 × Huffman	POPX	1966
SC285	Latham's double	OP	1966
SC332	T115 × GT106	2X	1966
SC344	W Tux × Latm's D × PEE DEE 5	POPX	1966
SC353	SC composite	COMP	1966
SC359	16 Line multiple	SYN	1966
SC375	SC composite	SYN	1966
SC301D	SC composite	COMP	1967
SC333	T115 × white tuxpan	POPX	1967
SC213W	(GT112 × NC33) GT112	BC	1971

continued

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
SC235	F3 × Huffman	POPX	1974
SC13W	GT112 Der	Inbred	UN
South Dakota			
SDp316	DSp236 × K63	2X	1975
SDp317	SDp236 × K63	2X	1975
SD104 (germplasm)	(W182B × SD316W)W182B/9	BC	1988
SD105 (germplasm)	(W117 × SD316W)W117/9	BC	1988
SD106 (germplasm)	(A641 × SD316W)A641/9	BC	1988
SD107 (germplasm)	(A634 × SD316W)A634/9	BC	1988
SD108 (germplasm)	(B73 × SD316W)B73/9	BC	1988
SD50	(W153R × SD316W)W153R/9	BC	1988
SD53	(A632 × SD316W)A632/9	BC	1988
SD55	(ND248 × SD316W)ND248/9	BC	1988
SD59	(A619 × SD316W)A619/9	BC	1988
SD66	(B84 × SD316W)B84/7	BC	1988
SD73	(SD33 × SD316W)SD33/7	BC	1988
SD54	(ND247 × DS316W)ND247/9	BC	1989
SD56	(ND250 × DS316W)ND250/9	BC	1989
SD57	(ND251 × SD316W)ND251/9	BC	1989
SD60	(W64A × SD316W)W64A/9	BC	1989
SD63	(CM105 × SD316W)CM105/9	BC	1989
SD65	(A654 × SD316W)A654/9	BC	1989
SD69	(A665 × SD316W)A665/9	BC	1989
Tennessee			
T13	Neal's paymaster	OP	1957
T14	Neal's paymaster	OP	1957
T18	Neal's paymaster	OP	1957
T61	Neal's paymaster	OP	1957
T61WC	(T61 × Snelling S. S.)T61/6	BC	1957
T101	(WF9 × Oh51A)X FL 916	POPX	1959
T105	CI201H × YS66	2X	1959
T111	Jellicorse	OP	1959
T113	Jellicorse	OP	1959
T115	Jellicorse	OP	1959
T125	Huffman × Oh40B	POPX	1961
T127	T111(RBL × ILL.A) T111	BC	1961
T129	T113 (M14 × Oh51) T113	BC	1961
T131	(T13 × T103)T13/2	BC	1961
T133	Jellicorse (I205 × L289)	POPX	1961
T135	T115 (I205 × L289) T115/2	BC	1961
T137	T111 (RB893 × RB751) T111	BC	1961
T139	T115(Huffman × Oh40B)T115	BC	1961
T311	T111 (I205 × L289)	3X	1961
T337	T113 (RB893 × RB751)	3X	1961
T11 S (SW)	(K6/I0 × MIX)(H2/39)	3X	1963
T15 S (SW)	(H1/39)(H1 × 39/51)	2X	1963
T315	T111 (RB.L × ILL.A)	3X	1966

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
T331	T113 (I205 × L289)	3X	1966
T357	T115 (RB893 × RB751)	3X	1966
T393	T109 (M14 × Oh51)	3X	1966
T141	Huffman × RB751	POPX	1974
T143	(R7 × T61)T61W.C.	3X	1974
Ga209cms C	Ccms source X Ga209/	BC	1976
Ga209cms El. Sal.	El. Sal. Cms source × Ga209/	BC	1976
Ga209cms R	Rcms source × Ga209/	BC	1976
Ga209cms RB	RBcms source × Ga209/	BC	1976
K55cms J	Jcms source × K55/	BC	1976
Ky21cms J	Jcms source × Ky21/	BC	1976
Mo18Wcms C	Ccms source × Mo18W/	BC	1976
Mo18Wcms El. Sal.	El. Sal. cms source × Mo18W	BC	1976
Mo18Wcms J	Jcms source × Mo18W/	BC	1976
Mo18Wcms RB	RBcms source × Mo18W/	BC	1976
Mp339cms C	Ccms source × Mo18W/	BC	1976
Mp339cms RB	RBcms source × MP339/	BC	1976
T111cms RB	RBcms source × T111/	BC	1976
T139cms C	Ccms source × T139/	BC	1976
T139cms J	Jcms source × T139/	BC	1976
T139cms RB	RBcms source × T139/	BC	1976
T139cms S	Scms source × T139/	BC	1976
T61W.C.cms El. Sal.	El. Sal. cms source × T61W.C./	BC	1976
T145	TSGP	SYN	1979
T147	TSGP	SYN	1979
T149	Jellicorse × Teko yellow	POPX	1979
T151	Jellicorse × Teko yellow	POPX	1979
T153	Jellicorse × Teko yellow	POPX	1979
T155	Jellicorse × Teko yellow	POPX	1979
T157	(T115 × T111)T115/9	BC	1979
T157A	(T115 × T111)T115/9	BC	1979
T159	TLLE-C10	SYN	1979
T161	TLLE-C10	SYN	1979
T163	Waverly Vir res dent syn	SYN	1985
T165	(T232 × GA209)T232	BC	1987
T167	Mo17 × CI66	2X	1987
T169	(T226 × Mo18W)T226	BC	1989
T171	(((B73 × CI66)F2)XB73)S6	BC	1991
Ky21cms 474	474cms source × Ky21/	BC	UN
Ky21cms C	Ccms source × Ky21/	BC	UN
Ky21cms CA	CAcms source × Ky21/	BC	UN
Ky21cms CHAPAL-OTE	CHAPALOTEcms × Ky21/	BC	UN
Ky21cms D	Dcms source × Ky21/	BC	UN
Ky21cms EK	EKcms source × Ky21/	BC	UN
Ky21cms EP	EPcms source × Ky21/	BC	UN
Ky21cms G	Gcms source × Ky21/	BC	UN
Ky21cms HASTINGS	Hastings cms × Ky21/	BC	UN
Ky21cm I	Icms source × Ky21/	BC	UN

continued

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
Ky21cms IA	IAcms source × Ky21/	BC	UN
Ky21cms K	Kcms source × Ky21/	BC	UN
Ky21cms M	Mcms source × Ky21/	BC	UN
Ky21cms ME	MEcms source × Ky21/	BC	UN
Ky21cms ML	MLcms source × Ky21/	BC	UN
Ky21cms MY	MYcms source × Ky21/	BC	UN
Ky21cms R	Rcms source × Ky21/	BC	UN
Ky21cms RB	RBcms source × Ky21/	BC	UN
Ky21cms S	Scms source × Ky21/	BC	UN
Ky21cms SD	SDcms source × Ky21/	BC	UN
Ky21cms TA	TAcms source × Ky21/	BC	UN
Ky21cms VG	VGcms source × Ky21/	BC	UN
Ky21cms W	Wcms source × Ky21/	BC	UN
Texas			
Tx158	White source	UN	1939
Tx3A	White source	UN	1939
Tx4R	White source	UN	1939
Tx102A	Blue grain	OP	1956
Tx155A	Blue grain	OP	1956
Tx4R3	Surecropper	OP	1956
Tx61M	Surecropper	OP	1956
Tx585	Pirater white dent	OP	1960
Tx102ATcms	Tx102A(Tcms)	BC	1968
Tx155ATcms	Tx155A(Tcms)	BC	1968
Tx585Rf	Tx585(Rf)	BC	1968
Tx61MRf	Tx61M(Rf)	BC	1968
Tx29A	Tx61M × Tx325	2X	1974
Tx29Ac (cms)	Tx29A-sterile	Inbred	1974
Tx80	6250 white composite	COMP	1981
Tx601W	Tx601 white conversion	UN	1984
Tx601W"C"st (cms)	Tx601 white conversion	UN	1984
Tx71	(Ky27 × K55) × Tx582	3X	1984
Tx81	(Ky27 × K55) × Tx582	3X	1984
USDA			
A61	White source	UN	1938
A62	White source	UN	1938
A63	U.S. ILA	UN	1938
A64	U.S.41	4X	1938
A65	U.S.61	4X	1938
A66	U.S.43	4X	1938
A67	UN	UN	1938
A68	U.S.11B	4X	1938
CI11B	Boone Co. white	OP	1948
CI23	Boone Co. white	OP	1948
CI24	Boone Co. white	OP	1948
CI41	Boone Co. white	OP	1948
CI43	Boone Co. white	OP	1948

TABLE 8.3 (CONTINUED)
White Endosperm Inbred Lines Released from Different U.S. Breeding Programs[a]

Name[b] and origin	Pedigree	Source type[c]	Year released
CI61	Boone Co. white	OP	1948
CI64	(K64 × Mo21A)K64/3	BC	1955
CI49A	Mo21A × Ky49/4	BC	1958
CI49B	Mo21A × Ky49/4	BC	1958
CI66	L97 × K55/2	BC	1958
CI127	Ky27 × L97	2X	1961
CI64cmsRfRf	CI64	BC	1964
CI64rfrf	CI64	BC	1964
USDA-SEA			
GT202wx	(GE19 × wx38–11)/6	BC	1974
GT206wx	(L501 × wx38–11)/6	BC	1974
GT209wx	(SC335 × wx38–11)/6	BC	1974
Virginia			
Va1	Rose white dent	OP	1959
Va2	Rose white dent	OP	1959
Va3	(H21 × T61) T61	BC	1959
Va4	(H21 × K55) K55	BC	1959
Va5	(T61 × K55) K55	BC	1959
Va6	(K41 × K44)K44/2	BC	1959
VA7	(Ky122 × K44)K44	BC	1959
Va8	Johnson Co. white	OP	1959
Va9	Johnson Co. white	OP	1959
Va10	M14 × K64	2X	1959

[a] Name and descriptor information obtained from Henderson;[61–70] reports of the North Central Corn Breeders Research Conference, the North Eastern Corn Improvement Conference, and the Southern Corn Improvement Conference; the National Germplasm Resources listing; and release statements or registrations.
[b] FL = Flint; POP = popcorn; SW = sweet corn; o2 = opaque-2; wx = waxy; Ga-s = gametophytic control; cms = cytoplasmic sterile; rf = sterility nonrestorer; Rf = sterility restorer; germplasm = germplasm release.
[c] 2X … 4X = single cross … Double cross; BC = backcross; COMP = composite cross; INBRED = inbred segregate; OP = open-pollinated variety; POPX = crosses of variety or variety × inbred; SYN = synthetic variety; UN = source type, pedigree, or year of release unknown.

of release for some entries in Table 8.3 are approximate because many of their earlier releases were not publicly registered or otherwise announced. Some pedigrees are not available or were abbreviated so that an approximation of the breeding procedure used by the originating breeder was sometimes made by this author. Because some errors, as suggested above, are undoubtedly associated with the inbred list, the following summarization is not exact, but in this author's opinion, does represent the general trend.

Of the 465 inbreds listed in Table 8.3, State Agricultural Experiment Stations, USDA research locations, and one foundation seedstock organization released a minimum of 36 white inbreds per complete decade since 1931 (Table 8.4). The number of white inbreds released in the 1971 decade is somewhat inflated in that many releases were multiple conversions of few lines to different cytoplasmic male sterility sources. The listing of states that released white inbreds in Table 8.4 identifies those states with active breeding programs during the time periods indicated. Because releases of inbreds by State Agricultural Experiment Stations in the early years were sometimes restricted to seed producers or farmers in that state, inbred releases may not have

TABLE 8.4 NUMBER OF RELEASED WHITE ENDOSPERM INBREDS SUMMARIZED BY DECADE AND STATE

Decade	Number	State
1931–1940	36	IN, KY, TX, UDSA
1941–1950	43	IL, IN, KS, MA, MN, USDA
1951–1960	84	IA, IL, IN, KS, KY, LA, MO, MN, NC, NY, TN, TX, USDA, VA
1961–1970	69	GA, IN, KY, MO, MS, SC, TN, TX, USDA
1971–1980	103	AR, GA, IL, IL-IFS, IN, KY, KS, LA, MO, MS, SC, SD, TN, TX, USDA
1981–1990	54	GA, IN, IL-IFS, MS, NC, ND, SD, TN, TX
1991–	1	NC
Unknown	69	IL, FL, NE, NC, SC, TN
Total	459	

been recorded, and the names of some states may have been omitted. The summarization in Table 8.5 shows the number of inbreds released by each of the 24 state, USDA, and seedstock organization breeding programs. Most white inbreds originated in southern and western U.S. Corn Belt states. A central U.S. Corn Belt state with a large number of releases was Illinois, but most were released before 1960.

TABLE 8.5
Number of White Endosperm Corn Inbreds Developed by State, Federal, and Foundation Seedstocks Breeding Programs in the U.S.

State	Inbreds released
Arkansas	2
Florida	4
Georgia	7
Iowa	9
IFS	22
Illinois	45
Indiana	29
Kansas	18
Kentucky	34
Louisiana	14
Massachusetts	1
Minnesota	14
Mississippi	5
Missouri	53
North Carolina	29
North Dakota	1
Nebraska	5
New York	3
South Carolina	13
South Dakota	20
Tennessee	84
Texas	19
USDA	24
Virginia	10
Total	465

White inbreds have been derived from segregating sources as basic as open-pollinated varieties to sources as complex as specifically derived synthetic varieties (Table 8.6). The initial breed procedure, selfing with selection (pedigree selection or the inbred-hybrid method of Shull[45]) in an open-pollinated variety, accounted for about 31% of all white inbreds. These inbreds were classified as first-cycle inbreds by Jenkins[48] because they were direct isolations from a segregating population that had not been subjected to prior inbreeding and selection. The use of single cross, three-way cross, double-cross, double-double cross, backcross, and inbred (segregates from an inbred line) source populations to recycle or include desirable traits from other highly selected inbreds also prevailed as a source of inbreds. These were considered as second- or higher-cycle lines because specific traits or qualities that were incorporated into the improved white inbreds had been previously isolated in other inbreds. The backcross procedure has been particularly important for white corn breeders because many significant yield advances were first made or incorporated in yellow endosperm corn. Subsequently, backcrossing was used to transfer the desirable traits to white endosperm types. Bauman[54] suggested that one or two backcrosses followed by selection were more appropriate than continued backcrossing to recover the entire recurrent parent. This modification of the backcross procedure allowed for the selection of potential transgressive segregates that might be eliminated by excessive backcrossing. Other uses of backcross breeding have been to incorporate male sterile cytoplasms, genes for improved protein or starch quality, the gene for gametophytic transmission, and other factors into existing white inbreds. This group of lines derived from recycling procedures accounts for about 50% of the released white endosperm inbreds (Table 8.3). Unfortunately, the use of yellow endosperm, exotic, and other white inbred genotypes often affects the desired white endosperm and cob or kernel type of the recurrent parent and can complicate the recovery of a desirable white endosperm type. Although not unavoidable, such complications can increase the time required for white endosperm inbred conversions or isolations. In some instances, these undesirable genes or alleles can cause inadvertent fixation of traits that can lead to dead ends, i.e., a dingy white or pale lemon yellow endosperm, a latent gene for anthocyanin pigmentation (sun red), or perhaps a viviparous or virescent gene or allele. Genetic explanations for many of these complications are discussed in the white corn inheritance section.

Recently, recycling of previously selected white endosperm inbreds has included formation of genetically variable source populations from existing white inbreds or varieties. Exotic germplasm

TABLE 8.6
Number of White Endosperm Inbreds Released by Public State or Federal Breeding Programs in the U.S. Summarized by Source Type

Source type	Number	States
Open-pollinated population	143	GA, IA, IL, IN, KS, KY, LA, MN, MO, MS, NC, NE, NY, SC, TN, TX, USDA, VA
2×	52	GA, IFS, IN, KS, KY, LA, MN, MO, SC, SD, TN, USDA, VA
3×	13	IFS, KS, ND, TN, TX
4×	5	KS, USDA
8×	1	NC
Backcross	149	GA, IFS, IL, IN, KS, KY, MN, MO, SC, SD, TN, TX, USDA, VA
Inbred	14	KY, MO, NC, SC, TX, USDA
Population cross	32	IL, IN, KS, KY, MA, MN, MO, MS, SC, TN
Composite population	9	IA, MN, SC, TX
Synthetic	22	AR, IL, IN, KY, MO, SC, TN
Unknown	25	IN, FL, KY, LA, TX, USDA
Total	465	

has also been included to increase genetic diversity. These new populations are labeled in Table 8.3 as composite populations, population crosses, and synthetic populations. Only about 13% of the inbreds in Table 8.3 were derived from this group of source populations. These procedures have not received as much attention with white endosperm corn as with yellow endosperm corn, but may contribute significantly in future development. Hallauer et al.[52] and Hallauer and Miranda[53] reviewed the applicable population improvement procedures in-depth.

The general trends, indicated in Table 8.6 and this author's interpretation, are that few if any future white inbreds will be derived by pedigree selection from open-pollinated varieties. Recycling of the better white inbreds will continue, and elite yellow inbreds and their heterotic patterns will be adapted for use as white endosperm corn. Backcrossing and selfing within F_2 populations of inbred lines will be very common, and the use of synthetic populations may increase, but only if substantial effort is made to increase overall productivity levels of the synthetic populations. White corn does not have a unique heterotic pattern as has been found for yellow endosperm corn. This conclusion might have been reached in the 1930s when white hybrids were made from inbreds of the same open-pollinated variety. Mungoma and Pollack[55] studied seven yellow and three white varieties and found no heterotic responses among the three white varieties nor among crosses of the three white varieties with the seven yellow varieties.

A comparison of white corn breeding methods with yellow corn breeding methods would show few differences. The differences that would become most evident are that white corn breeding requires different or additional emphases which are to be discussed. A second difference is not so much the type of breeding procedure as the amount of breeding effort. As yellow corn production became dominant over white and as corn breeding programs transferred from state or federal programs to private programs, the amount of effort expended on white corn breeding was severely reduced. Because private corn breeding programs must be supported by revenue from sales of seed corn, it is not surprising that private programs emphasize yellow corn breeding. Less than 1% of the current U.S. corn acreage (Table 8.1) is white corn; thus, less than 1% of seedcorn sales will be white seed corn. The corn breeding programs of many southern states, where white corn was emphasized or at least minimally covered, also declined in number during the last several decades even though white corn was still produced and encouragement for breeding research was being provided by a few white corn dry millers. A resurgence of white corn breeding, however, began in 1977 when a unified group of white corn millers, the American Corn Millers Federation, and the Quaker Oats Company, provided operating funds for several State Agricultural Experiment Station white corn breeding programs and for a national testing program of newly developed white endosperm corn hybrids. Both large and small dry millers and some seed companies contributed funds. The breeding and testing efforts have been successful in improving yield of white corn hybrids. Between 1977 and 1991, the average yield of new white hybrids tested in the White Food Corn Performance Tests[6–20] has increased at a rate of 3.2 bu/a/year (Figure 8.1).

Contemporary white corn breeding differs from yellow corn breeding primarily in that the selection criteria are determined by the end product user. As white corn utilization has shifted from animal feeding to specialized human food and industrial products from dry milling, high grain quality has become equally as important as increased grain yield and disease or insect tolerances related to grain yield potential. High quality, white endosperm corn must have the bright white color that can be obtained only with the *y* allele in homozygous state plus the absence of factors that modify *y* and factors that contribute kernel anthocyanin pigmentation. White cob must be obtained from the homozygous condition of the *P-ww* allele. The nonbrowning silk allele, *P-www,* would be preferred, although earworm feeding resistance may be an overriding factor. If possible, the white endosperm genotype should have the colorless *r-g b* homozygous genotype that would provide further protection against anthocyanin pigmentation.

In addition to the cob and kernel color requirements, high quality white endosperm corn should have large, uniform, dense, and nondented or only slightly dented kernels. The large, uniform, dense kernels provide a kernel type from which less fine flour and more large grits can be extracted

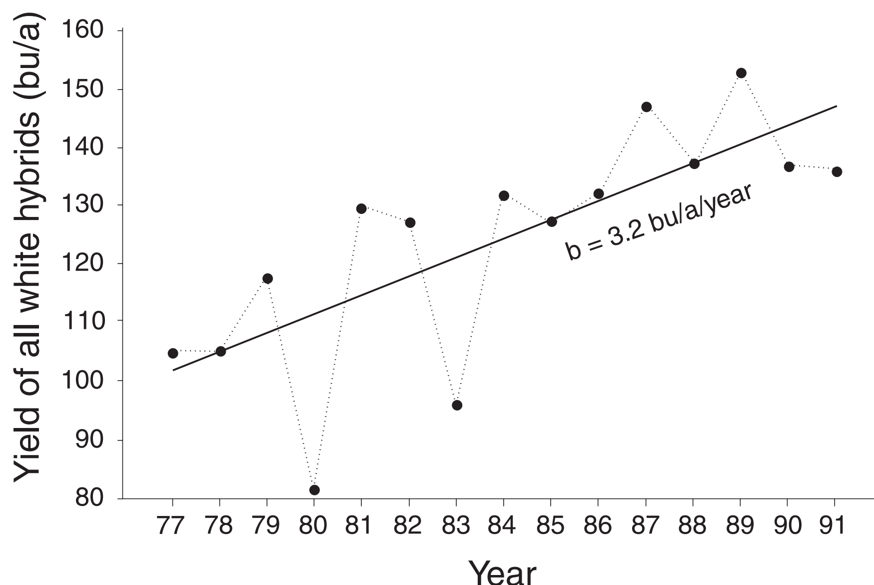


FIGURE 8.1 White Food Corn Performance Test Yields[6–20] from 1977 to 1991. Data are obtained from national tests conducted as part of the National White Food Corn Test program and sponsored by the American Corn Millers Federation.

by dry millers. A nondented kernel indicates that little soft starch is present and fine flour particles will be minimal from dry milling. Kernels without stress cracks also provide a greater yield of large grits. Although stress cracks are known to be caused by harvest and drying conditions, some genotypes are more or less susceptible.[9,10] Pericarp that is easily separated from endosperm is also a valuable trait for dry milling[56] as well as for processing of corn for masa flour.[57] Because processing procedures differ for the two usages, the ease of pericarp removal for each process may be influenced by different genetic factors.

Disease-free grain is essential for high quality white corn. Grain is less likely to have fungal pathogens if it has not been damaged by insects, birds, or other pests, has not lodged and made contact with the soil, and has not been damaged or had trash introduced during harvest by improperly adjusted harvest equipment. Ear rots of corn in the field that are found most frequently or have the greatest consequences are Fusarium ear rot (*Fusarium moniliforme*), Diplodia ear rot (*Diplodia maydis*), and Aspergillus ear rot (*Aspergillus flavus*).[58] Genetic variation for susceptibility has been reported for Fusarium and Diplodia ear rots. Field susceptibility to Diplodia has been associated with upright ears and incomplete husk cover. Incomplete husk cover is also a factor in insect and bird damage, which in turn can introduce or provide convenient access to kernels by pathogenic fungi. Darrah et al.[59] and Scott and Zummo[60] determined that resistance to *Aspergillus* fungi, which produce carcinogenic aflatoxins, is present in the corn germplasm. Inoculation methods and analytical procedures are improving so that evaluations of breeding selections for *Aspergillus* susceptibility may soon be practical. Stress as provided by high temperature, drought, and leaf-blighting fungi may also increase the susceptibility of corn plants to *Aspergillus* and other fungi; thus, corn hybrids that are more tolerant of these stress factors will minimize fungal problems. Fusarium, Diplodia, and other fungi also cause stalk rot in susceptible plants. Although the stalk-rotting phase of these pathogens may not directly affect the grain, grain

quality may be influenced indirectly by increased stalk lodging which can increase ear-to-soil contact and trash collected with shelled grain. Other ear and kernel rots affect corn, but are found less frequently.[58] Numerous storage molds, including *Aspergillus* spp, may also influence white corn quality, and are a function of storage conditions, grain moisture content, and temperature.[58] Most fungi that attack corn kernels in field situations are generally not a problem in stored grain. Grain moisture contents below 14.0% inhibit most fungal growth. Because moisture contents this low require extensive drying, a grain producer may assume that a higher moisture content is economically acceptable. For white corn storage, however, this may create significant problems because some *Aspergillus* species (*A. glaucus* and *A. restrictus*) will grow slowly at 14 to 14.5% moisture. *A. flavus* grows only at moisture contents, above 16% and temperatures above 40 to 45°C, but pockets of trash, broken kernels, or high moisture grain may provide or contribute to these conditions. For reasonably safe grain storage, a cautious grower would be better off to err on the low side, with lower storage moisture, and to carefully monitor stored grain. Breeding for hybrid resistance to storage molds is not practical at this time due to the lack of usable screening methods and the large number of different fungi involved.[58]

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9 Baby Corn

Chokechai Aekatasanawan

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I. INTRODUCTION

Baby corn is the young and unfertilized ear of the corn (*Zea mays* L.) plant harvested when the silks have either not emerged or just emerged (1 to 3 cm). The husked young ear in canned or fresh ear style is a more popular vegetable because of its sweetness, flavor, and crispness. Generally, the requirements of baby corn for the fresh market or processing are (1) ear size of 4 to 9 cm length and 1.0 to 1.5 cm diameter and (2) good quality, i.e., yellow color, straight ovary row arrangement, unfertilized and unbroken ear, and size within factory specifications. For baby corn production, varieties are grown under high plant densities (120,000 to 160,000 plants ha^{-1}), irrigation and high nitrogen application. Someone might use low plant density for picking the first ear for baby corn and the second ear as sweet or field corn.

Baby corn is a highly nutritious vegetable. Its nutritional value compared with other vegetables (cauliflower, cabbage, tomato, eggplant, and cucumber) is shown in Table 9.1, according to Yodpet.[1] Bar-Zur and Schaffer[2] reported total sugar content of baby corn observed in all genotypes studied ranged from 20 to 30 mg g^{-1} fresh weight and the major sugars were the reducing sugars, glucose and fructose, in about equal amounts of 10 to 15 mg g^{-1} fresh weight. Sucrose was present in only small amount at silking. Moreover, the content of 17 amino acids and nine essential amino acids, including lysine, was found in baby corn (Yu et al.[3]).

TABLE 9.1
Nutritional Value of Baby Corn from Analysis of 100 grams Compared with Other Vegetables

Components	Baby Corn	Cauliflower	Cabbage	Tomato	Eggplant	Cucumber
Moisture (%)	89.10	90.30	92.10	94.10	92.50	96.40
Fat (g)	0.20	0.04	0.20	0.20	0.20	0.20
Protein (g)	1.90	2.40	1.70	1.00	1.00	0.60
Carbohydrate (mg)	8.20	6.10	5.30	4.10	5.70	2.40
Ash (g)	0.06	0.80	0.70	1.60	0.60	0.40
Calcium (mg)	28.00	34.00	64.00	18.00	30.00	19.00
Phosphorus (mg)	86.00	50.00	26.00	18.00	27.00	12.00
Iron (mg)	0.10	1.00	0.70	0.80	0.60	0.10
Vitamin (fu)	64.00	95.00	75.00	735.00	130.00	0.00
Thiamine (mg)	0.05	0.06	0.05	0.06	0.10	0.02
Riboflavin (mg)	0.08	0.80	0.05	0.04	0.05	0.02
Ascorbic acid (mg)	11.00	10.00	62.00	29.00	5.00	10.00
Niacin (mg)	0.03	0.70	0.30	0.60	0.60	0.10

Source: Yodpet, C. Studies on sweet corn as potential young cob corn (*Zea mays* L.), Ph. D. thesis, University of the Philippines. Philippines, 1979.

Another benefit to baby corn production has been integrated with dairy farms in Thailand because only 13 to 20% of fresh ear weight is used as human food. The rest (husk and silk) can be used as green herbage for ruminants and pigs. Faungfupong et al.[4] reported that it could produce 40 to 43 t ha^{-1} of plant fresh weight. Out of this amount, 6 to 7% was attributed to the detasseling plant part, 8 to 10% to the husk and 83 to 86% to the remaining plant parts after ear harvesting. These plant materials are highly nutritive and can be used as roughage or silage for beef cattle and dairy cow. Cheva-Isarakul and Paripattananont[5] found that baby corn waste contained 86.4% moisture, 94.4% organic matter (OM), 10.6% crude protein, 55.1% neutral detergent fiber (NDF), 26.8% acid-detergent fiber (ADF), and 2.0% acid-detergent lignin. Digestibilities of DM, OM, nitrogen-free extract, NDF, and ADF, were over 70%.[5] The nutritive value of baby corn residue obtained after the harvest for 1, 5, 10, and 15 days contained 18.9, 19.3, 20.1, and 21.3% dry matter; 9.9, 8.8, 8.7, and 8.4% protein; and 23.3, 26.6, 29.0, and 29.9% crude fibre, respectively.[6] Chunjula[6] indicated that the baby corn residue was a good quality roughage for the beef cattle compared with Guinea grasses. Snitwong and Rungroung[7] showed a significant difference ($P < 0.05$) between para grass and baby corn husk; the value being 7.97 vs. 10.18 kg/head/day of 2,375.53 vs. 3,105.22 kg for the production of milk yield at 305 days. Both had nonsignificant differences in protein and lactose. They indicated that income from baby corn wastes (stover, husk, and stover and husk) was higher than from para grass.

II. TYPES OF GERMPLASM USED IN BREEDING

Sweet corn, field corn, popcorn, silage corn, etc. grown as food or feed can be used as germplasm sources for baby corn breeding. However, only specific varieties will be used for baby corn production for the fresh market or processing. Bar-Zur and Schaffer[2] showed that there was no significant increase in sugar content of baby corn ears attributable to the *su*, *se,* or *sh2* genes compared with *Su*. Types of germplasm used in breeding should be considered for traits as follows: (1) high yields of unhusked, husked, and standard or good ear weights, (2) high husked and unhusked ear weight ratio, (3) yellow cob with beautiful shape, straight ovary row arrangement, and small ovary, (4) prolificacy, (5) early variety, (6) tolerance to higher plant density, (7) good

root and stalk strength, (8) tolerance to leaf diseases and insects at vegetative growth, and (9) response to N fertilizer.

From reviewed literature, most baby corn breeding programs have been extensively developed in Thailand, and source materials used have been mainly developed by Kasetsart University, Department of Agriculture, and private seed companies as follows:

1. Open-pollinated varieties:
   - 1.1 Field corn varieties: Suwan 1,[8] Suwan 2,[8] and Suwan 3,[9] These are resistant to corn downy mildew (DMR) caused by *Peronosclerospora sorghi.*
   - 1.2 Sweet corn variety: Thai Supersweet Composite 1 DMR.[10]
   - 1.3 Baby corn varieties: Rangsit 1,[11] Baby Corn Thai Composite 1 DMR,[12] Chiangmai 90,[13] and Kasetsart 1.[14]
2. Field corn inbreds: Ki3, Ki11, Ki20, Ki21, Ki27, Ki28, Ki36, and Ki37. These Kasetsart inbreds are DMR inbreds, and they were released between 1982 and 1997.[15]
3. Baby corn inbreds: Ki39 (Rangsit 1(H)C1-S_8-5) and Ki40 (Suwan 2(S)C7-S_8-2-4). Both were released in 1992.[15]
4. Baby corn hybrids: PACB 421, PACB 116, PACB 129, and PACB 444 from Pacific Seeds Co., Thailand; G-5414, NTB 017, and NTB 048 from Novartis (Thailand) Co.; and Uniseed B-50 from Uniseeds Co., Thailand. PACB 421 and G-5414 are very popular for the fresh market and processing in Thailand, and they are exported to some countries.

Other sources of DMR which can be used for baby corn breeding are CIMMYT's lowland tropical germplasm; e.g., Population 28 (Amarillo Dentado), Population 31 (Amarillo Cristalino-2), etc.[16]

Prolific inbreds, hybrids, and populations of field corn are potential sources of germplasm for baby corn breeding programs; for example, baby corn prolific hybrids (i.e., NY 569 and NY 573) developed by Bar-Zur and Saadi[17] at Newe Ya'ar Experiment Station, Israel.

Another interesting source for baby corn breeding developed by Galinat[18,19] and Galinat and Lin[20] at University of Massachusetts, U.S. is silkless baby corn, which cannot be pollinated and remains soft and edible. Galinat used two different recessive genes for the tassel-seed trait (*ts2* and *ts1*) on chromosomes 1 and 2, respectively. These genes are restorers for the silkless gene, *sk*, also on chromosome 2. The double mutant, *sksk ts2ts2* and *sksk ts1ts1* with selection for normal sexual balance, function as normal corn. The double mutant hybrid *ts2Ts2 ts1Ts1 sksk* is completely silkless.

III. BREEDING METHODS USED

Baby corn breeding can use many of the techniques and theories developed by field corn breeders.[21] However, it is different in practice because of the different end use of the final product which is highly perishable. Crispness of baby corn declines with time after silking within 4 days depending on varieties (Table 9.2).[17]

A. Inbred and Hybrid Development

1. Inbred Development

Pedigree selection is the most commonly used method for inbred development from the germplasm sources (e.g., OPVs, synthetic varieties, and single cross, three-way cross, and double cross hybrids), and from related line crosses of field corn or sweet corn considered to have high potential for prolificacy. These germplasm sources have to be selected under higher population density to put more stress. Visual selection is effective during inbreeding under the stress, especially for important traits; e.g., stalk and root strength, early silk emergence, prolificacy, and shorter plant height. Other

TABLE 9.2
The Effect of Harvest Date on Crispness[a] of Unfertilized Baby Corn Ears

	Days after Silking			
Cultivar	**0**	**2**	**4**	**6**
Classic R32 (*suse*)	4.0 a[b]	4.0 a	3.0 ab	2.1 b
Jubilee (*su*)	3.9 a	3.7 a	2.9 ab	2.7 a
NY 573 (*Su*)	4.0 a	3.9 a	3.7 a	2.7 a
SUM SW 7200 (*sh2*)	4.0 a	2.9 b	2.6 b	1.6 b

[a] 4, crisp and fragile; 3, crisp, acceptable; 2, tough; 1, very tough.
[b] Mean separation within columns by multiple range test, $p = 0.05$.

Source: Bar-Zur, A. and Saadi, H., in *J. Hort. Sci.,* 65, 97, 1990.

characters should also be considered, such as seedling vigor, insect and disease resistance, pollen shedding, and plant and ear aspects, as is practiced in field corn.[22,23] The effectiveness of selection among and within progenies depends on the relative heritability of traits selected. Screening techniques for the important pests should be developed and used for selecting the tolerant or resistant lines; for example, the success in selection for DMR in artificial blocks in Suwan 1, Suwan 2, and Suwan 3 varieties in Thailand.[8,9] Pedigree selection can be modified with a conventional backcrossing program to add or recover some interested traits; e.g., prolificacy, DMR, etc.

Prolificacy in field corn[24] can be applied to baby corn breeding. It is not clear that prolificacy is controlled by partial to complete dominant[25,26] or recessive[27–31] genes. Furthermore, opinions differ as to whether number of ears is polygenetically controlled[32] or is more simply inherited.[31] However, most authors agree that gene action for controlling prolificacy is mainly of the additive type.[26,33–37] Hallauer[29] suggested that prolificacy fits the description of a threshold trait in that the inheritance is quantitative but expression is qualitative. Duvick[30] demonstrated that multi-earedness traits can be transferred from a prolific to a nonprolific inbred line by continuous backcrossing. This result can be taken as evidence that a small number of genes can exert control over prolific potential in corn. Harris et al.[31] provided evidence that genotypic differences between one-and two-eared hybrids are affected by two loci with major effects.

2. Hybrid Development

Selected lines during inbreeding in baby corn should be evaluated in testcrosses in the S_3 or S_4 generations, as is practiced in field corn.[23] However, there is limited information available on choice of testers, heterosis, and heterotic patterns for baby corn, compared with field corn.[21,38,39] Practically, the use of tester depends on the breeding objectives. If the final hybrids are single crosses and three-way crosses, the testers should be an inbred and single cross, respectively. Heterosis in baby corn should be considered for prolificacy and other important traits, except for the fast development of young ears that is desirable for high grain yield in field corn. Hybrid development of baby corn has been conducted at Kasetsart University,[11,40–45] the Department of Agriculture,[46] and Maejo University,[47] Thailand; Newe Ya'ar Experiment Station,[2,17] Israel; Instititute of Agricultural Science Research, Hebei Province[48] and Yantai City Institute of Agricultural Science,[3] China. Other hybrid research has been done in Hungary, Vietnam, India, and Philippines. Bar-Zur and Saadi[17] showed that prolific baby corn hybrids, NY 569 and NY 573, gave the first baby corn ear on the stalk to reach the maximum size acceptable by consumers, and the successive ears were also within the size limits acceptable by the market (Table 9.3). The ears picked 3 to 4 days after silking of the first ear had a similar taste and texture to those of sweet corn or field corn.[17]

TABLE 9.3
Baby Corn Ear Size in Relation to Time from Silking and Location on the Stalk, Eden 1988

	Length (cm)			Diameter (mm)			Weight (g)		
Days after Silking[a]	**0**	**2**	**4**	**0**	**2**	**4**	**0**	**2**	**4**
				'NY-569'					
Ear I	7.8	8.3	9.8	8.7	9.0	11.3	3.6	4.9	9.6
Ear II	6.8	7.5	10.0	8.8	8.6	11.5	3.2	3.9	9.9
Ear III	6.4	6.5	7.0	6.8	7.0	8.0	1.5	2.3	3.3
SE	0.58	0.34	0.32	0.26	0.24	0.24	0.09	0.17	0.31
				'NY-573'					
Ear I	8.5	11.0	11.1	9.8	11.9	12.3	5.4	10.9	12.7
Ear II	7.1	9.9	9.9	9.3	10.2	10.7	3.6	7.8	10.5
Ear III	5.7	6.5	7.8	6.5	6.8	7.3	1.4	2.3	4.1
SE	0.52	0.61	0.59	0.22	0.24	0.29	0.33	0.28	0.41

Note: The results are average of all baby corn ears, which were harvested in subplots 1 m long, including ears that did not fit marketable criteria.

[a] Days after silking counted from silking of the first female flower.

Source: Bar-Zur, A. and Saadi, H., in *J. Hort. Sci.,* 65, 97, 1990.

Trakoontiwakorn et al.[41] studied heterosis in baby corn using nine inbred lines from five different sources of germplasm (i.e., Suwan, CIMMYT, Hawiian Sugar Super Sweet, Tropical Corn Belt Stiff Stalk, and Tropical Corn Belt Lancaster) for making diallel cross. They found that inbred lines from Suwan, CIMMYT, and Tropical Corn Belt Stiff Stalk crossed with Hawiaan Sugar Super Sweet gave heterosis for unhusked yield range 32 to 88% over Suwan 2(S)C7, an OPV check. Inbred lines from Suwan, CIMMYT, and Tropical Corn Belt Lancaster crossed with Hawiian Sugar Super Sweet had heterosis for husked yield range 78 to 130%. Suwan inbreds, however, showed good heterosis (260 to 335%) for standard ear yield, when mated with inbreds from CIMMYT and Tropical Corn Belt Stiff Stalk.

Line *per se* and topcross methods were compared for evaluating inbred lines for making baby corn hybrids by Sangtong et al.,[42] using 32 lines of Rangsit 1 (RS 1(H)C1-S_2) and 16 lines of Suwan 2 (SW 2(S)C7-F_2-S_6). Their results showed that the two evaluation methods were not correlated for the unhusked, husked, and poor ear weights, but the good ear weight had significant correlation ($r = 0.33$, $p = 0.05$) between the two methods. They indicated that the topcross method was more effective than the line *per se* method by using the good ear weight as criteria for selecting inbreds for making hybrids. They also reported that unhusked ear weight was positively correlated to husked and poor ear weights ($r = 0.64$ and 0.50, respectively, $p = 0.01$) but unhusked ear weight was not correlated with good ear weight.

The prediction of three-way and double crosses of baby corn using single crosses data from a diallel cross of 10 inbred lines extracted from SW 2(S)C7, (RS 1(H)C1), and Kasetsart inbreds (Ki) was investigated by Sittichoke et al.[43] They found positive correlation coefficients between predicted and actual three-way and double crosses when using data of the 1988 vs. 1989 early rainy seasons, within 1989 dry season, and 1989 dry season vs. average of three seasons, but not for the 1988 early rainy vs. 1989 dry seasons. They also showed that good ear weight was controlled by additive more than nonadditive gene action.

Sonwai et al.[44] reported the breeding of baby corn hybrids using 10 inbred lines from three sources of germplasm (i.e., four from SW 2(S)C7, RS 1(H)C1, and three from Kasetsart inbreds) for producing diallel crosses. Their result was in good agreement with Sittichoke et al.[43] that additive gene action was more important than nonadditive gene action for good ear weight. They also reported that SW 2(S)C7-F_2-S_6-28 and Ki28 gave high general combining ability (GCA). The single cross from both inbreds showed the highest yield for good ear weight of 354% over Suwan 2(S)C7. They also demonstrated that the highest yielders of three-way and double crosses produced good ear weights over the check by 418 and 332%, respectively.

Field corn inbreds for baby corn production were evaluated by Aekatasanawan et al.[45] They used 10 Kasetsart inbreds for making diallel crosses. Results indicated that Ki20, Ki21, Ki32, and Ki44 gave significantly positive GCA for husked ear weight and Ki20 and Ki36 for good ear weight. The highest yielders for good ear weight were Ki20 × Ki37 and Ki36 × Ki37.

For the hybrid breeding of baby corn at Chiang Mai Field Crops Research Center, Department of Agriculture, Thailand, Puddhanon et al.[46] used S_3 lines selected visually for plant type, uniformity, and yellow ear. Early generation hybrids were formed by crossing between the four selected lines in the 1990 dry season. They found that CMB 9002 and CMB 9001 hybrids produced the highest unhusked and husked ear weights, compared with Suwan 2. Their results showed that early, high-yielding, DMR baby corn hybrids can be derived from the Chiangmai 90 × Suwan 2 pattern. Puddhanon et al.[47] used baby corn Inbred Nos. 19-S_3#, 19-S_4, and 26-S_3# topcrossed with HY(7x8) single cross to produce three-way crosses and tested in four locations in 1994. They found that HY(7 × 8) × 26S_3# and HY(7 × 8) × 19S_3# gave average standard ear weights significantly higher than Chiangmai 90 variety.

B. Population Improvement

Population development and improvement using recurrent selection methods have been extensively used in field corn. Improved populations can be used as OPVs for farmers' use or potential germplasm sources for inbred development depending on the breeding objectives and stage of breeding programs. Limited information on population improvement is available. Most population improvement has been studied at Kasetsart University and the Department of Agriculture, Thailand.

The Department of Agriculture, Thailand initiated a baby corn breeding program in 1976.[11] The objective was to release a composite variety, with high yield, yellow color, good row arrangement, DMR, and wide adaptation. Various germplasm sources from India and Philippines were introduced. From 1977 to 1981, 147 field corn varieties were tested and selected at experiment stations and in farmers' fields. From this research, Ransit 1,[11,49] an OPV developed from [UPCA Var 1 × Cup FC Comp. DMR (F)C2] × D 745, was released in 1982 before the advent of the hybrid era. Then, it carried out baby corn improvement for earliness and high yield during 1986 to 1989 using germplasm evaluation, family selection, recombination of elite germplasm, random mating, and testing of F_1 to F_3 generations in multiple locations and farmers' fields.[46] And the Department of Agriculture released CMB 8704-F_3, the prolific and high-yielding OPV,[13, 46] in the name of Chiangmai 90.

Population improvement in Baby Corn Composite 1 DMR by S_1 and full-sib selections for yield and quality of young ear corn and DMR was conducted by Promson et al.[12] during 1987 to 1988 at the National Corn and Sorghum Research Center (Suwan Farm), Kasetsart University. Results revealed that all newly improved and original populations were not significantly improved in yield. After one cycle, S_1 recurrent selection showed higher potential for improving unhusked, husked, and good ear weights than full-sib selection. At the 10 and 20% selection intensities, the unhusked ear weight in the S_1 improved population was 9 and 16% higher than the initial population, while the husked ear weight was 7 and 10% higher, and the good ear weight was 24% higher. The 10 and 20% selection intensities for full-sib selection produced good ear weight 7% higher and

4% lower than the original population, while the husked ear weight was 2 and 0% higher, and the good ear weight was 10 and 7% higher. The average DM infection of the newly improved population was generally slightly higher than that of the original population. However, the DM infection of the improved population by the S_1 selection at 20% selection intensity was reduced by 6%. They also found that, over both S_1 and FS families, highly significant correlations (r) among unhusked, husked, and good ear weights, ranged from 0.36 to 0.90 ($p = 0.01$), and between the three traits and the total number of young ears, ranged from 0.15 to 0.74 ($p = 0.01$), except for the r value between the good ear weight and total number of ears for S_1 lines.

The improvement of baby corn for high yield and DMR was studied by Hankrirkkai[50] at Suwan Farm, Kasetsart University. RS1-KU1 and RS1-KU2 were recombined from 30 selected S_1 lines but from different groups. The first group produced higher standard ear weight but higher DM infection, whereas the second group was opposite to the first group. Results showed that when untreated with Apron 35 SD, RS-KU1 and RS1-KU2 had 43 and 35% DM infection, respectively, while its original variety, RS1(H)C1(MJ), had higher infection of 51%. All of the original and improved populations were not significantly different for unhusked, husked, and good ear weights in both untreated and treated conditions. However, under untreated condition, RS1-KU1 and RS1-KU2 produced 51 and 49%, respectively, higher ear weights than the original variety, while under treated condition, they gave only 41 and 25% higher ear weights than the original variety. RS1-KU2 was more resistant than RS1-KU1 in DM nursery, and its unhusked and standard ear weights were not different in both treated and untreated conditions.

From his study, the number of young ears per plant was highly correlated with unhusked, husked, good, and poor ear weights ($r = 0.806$, 0.678, 0.508, and 0.464, respectively, $p = 0.01$). Boonyalesnirun[51] studied the relationship between yield and agronomic characters of FS1 line of baby corn derived from the varietal cross (Suwan 3 × Baby Corn Composite 1 DMR). He showed that ear number per plant had a highly direct effect on good ear weight ($b_1 = 0.87$ and $r_{1y} = 0.79$, respectively). From reviews, it revealed that ear number per plant is the most important trait for improving good ear weight.[50,51] Thus, it should be used as selection index for baby corn improvement.

Selection for hybrids that respond to N fertilizer under higher plant density should be considered, because young cobs, fresh ear weights, number of ears per plant, percentage of acceptable cobs, and percent of sugar in young cob juice increased with increases in N-fertilizer rates up to the highest rate 160 kg N ha^{-1}.[52] The traits were not affected by P fertilizer.

IV. SOME PROBLEMS ASSOCIATED WITH BREEDING

Some problems associated with baby corn breeding can be summerized as follows:

1. There is limited information on baby corn breeding because few baby corn breeding programs have been actively working. Research and development on baby corn breeding were initiated in the late 1970s, compared with field corn and sweet corn breeding programs developed about 100 years ago.
2. There are limited germplasm sources developed for specific purposes of baby corn.
3. There is a lack of breeders in the limited baby corn breeding programs. Young staff or new breeders should be developed and encouraged in each breeding program.
4. It is time-consuming and labor intensive, because it requires detasseling, harvesting many times, and husking of green ears.
5. Harvest time cannot be delayed as with field corn, because green ears must be harvested when silk emerges 1 to 3 cm or 1 to 2 days. And they are husked within the harvested day.
6. Heterosis in grain yield and heterotic pattern in field corn cannot be applied to baby corn breeding, because it needs more small young ears per plant and slow development of young ears for high yield and high quality.

V. PRESENT AND FUTURE USES OF BREEDING

A. Present Use

1. Hybrids

At present, the target varieties of most baby corn breeding programs are superior, stable, high-yielding hybrids (single cross, three-way cross, and double-cross hybrids).[2,3,17–20,40–48] Most breeders have developed semiprolific or prolific inbreds from various germplasm sources of field corn and sweet corn for making prolific baby corn hybrids. Pedigree breeding with visual selection during line development is effective because of the relatively high heritability and additive effects of important traits in baby corn, especially for prolificacy, DMR, and resistance to root and stalk lodging. Baby corn hybrids have higher yield potential, better uniformity of silk emerged, shorter harvest, cob size, plant and ear heights, etc., compared with open-pollinated baby corn varieties (OPVs). Consequently, they have been used for the fresh market and processing and replaced OPVs very rapidly, particularly in this decade in Thailand.

2. Utilization of Male Sterility for Baby Corn Improvement

In baby corn production, the removal of tassels or detasseling is necessary for the stimulation of earlier harvest date, the enhancement of number of ears per plant or prolificacy, the increase of high yield, and the prevention of pollination. However, the detasseling results in higher cost and perhaps in yield loss affected by some leaf loss.[53]

a. Development and Evaluation of Male-Sterile Baby Corn Varieties

Aekatasanawan et al.[14,53,54] solved these problems by using C cytoplasmic male sterility to improve non-detasseled baby corn. They reported that during 1988 to 1991, six selected male-sterile (no anther exserted) lines from IITA (Nigeria) and Guatemala were used as females in crossing with two baby corn varieties: Suwan 2(S)C7 (SW2) and Thai Supersweet Composite 1 DMR (TSC 1 DMR). Both SW2 and TSC 1 DMR were backcrossed to Nigeria and Guatemala lines for five and two times, respectively. Suwan 2 had the same ancestor (Thai Composite #1) as Suwan 1. A breeding scheme for Suwan 1 and Suwan 2 varieties and germplasm assembled in Thai Composite #1 are shown in Figure 9.1 and Table 9.4, respectively.

The resulting five male-sterile varieties and three check varieties were evaluated in October 1991 at the National Corn and Sorghum Research Center (Suwan Farm), Kasetsart University, Nakhonratchasima, Thailand. A split plot design with detasseling and non-detasseling as the main plots and eight varieties as the subplots was employed. Population density was approximately 133,333 plants ha^{-1}. Results are shown in Table 9.5 and conclusions are as follows:

1. Two male-sterle varieties, MS (1, 2, 6, 7 × SW2) BC_5 and (CU88A(18x19) × SW2)BC_2, gave significantly higher unhusked and husked ear weights, ears per plant, and husked to unhusked ear weight ratio than the check, detasseled fertile Suwan 2.
2. For most characters the differences between the detasseled and non-detasseled of the two male-sterile varieties were not significant. However, the non-detasseled had significantly higher (p = 0.01) husked ear weight as well as higher husked to unhusked ear weight ratio than the detasseled.
3. In two detasseled fertile varieties, Suwan 2(S)C7 and Chiangmai 90, the detasseling gave significantly higher (p = 0.01) unhusked and husked ear weights, ears per plant, and husked to unhusked ear weight ratio than the non-detasseling.

The results of the detasseling and non-detasseling in the normal baby corn or the fertile baby corn supported the study of Grogan[55] on detasseling responses in corn as affected by climate, soil,

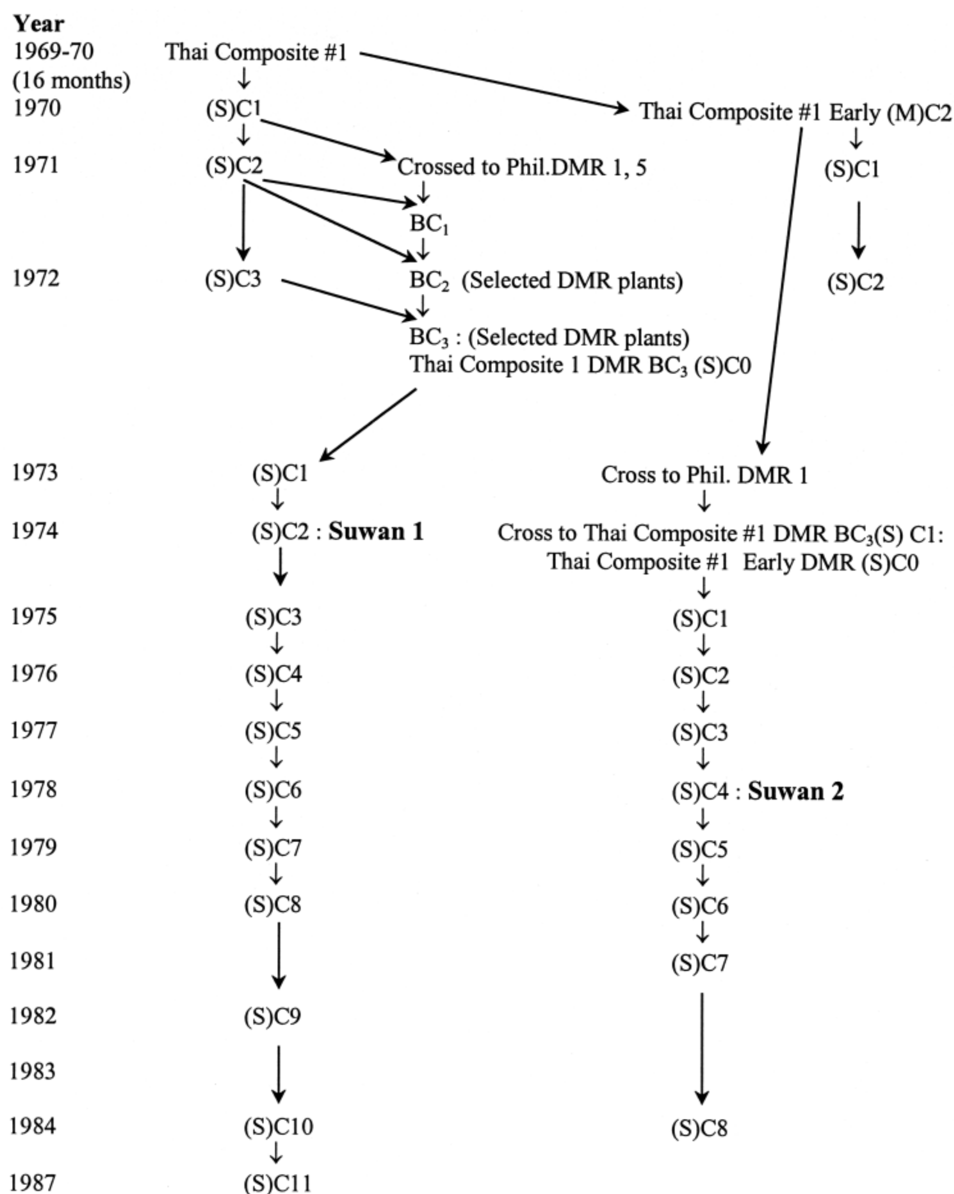


FIGURE 9.1 A breeding scheme for Suwan 1 and Suwan 2 varieties.

and competitive conditions. He concluded that under conditions of moisture and/or nutrient stress, yield increases associated with detasseling were due to the elimination of competition for nutrients between the ear and the tassel. He predicted that similar results might be expected if male-sterile cytoplasm were substituted for normal cytoplasm. Duncan et al.[56] and Hunter et al.[57] reported that the increase of grain yield from detasseling was larger and more consistent in higher plant densities resulting from the elimination of tassel light interception.

The results of detasseling and non-detasseling in the male-sterile baby corn supported the conclusions of Chinwuba et al.[58] that the male-sterile single cross outyielded their fertile counterparts at higher plant densities. And they reacted similarly to the the detasseled fertile single crosses at different plant densities when reductions in yield from mechanical injury and the initial stages

TABLE 9.4
Germplasm Assembled in Thai Composite #1

Source	Race	Variety
Caribbean Islands	Argentino	Cuba Gr.1
		Cuba 11J
		Puerto Rico Gr.
	Tuson	Cuba 40
	Argentino-Canilla-Criollo-Tuson	Cuba 1J
		Cuba V59
		Antiqua Gr.1
		Antiqua Gr.2
		Puerto Rico Gr.2
		Barbados Gr.1
		Cupurico
		Caribbean Flint Composite
		Composite Caribbean Amarillo
		Flint Composite Amarillo
		Tequisate Golden Yellow × Caribbean Composite
		Tequisate Golden Yellow × Guadalupe 12D-14D
Mexico and Central America	Tuxpeño	Veracruz 163
		Veracruz 181
		Veracruz Gr.48
		Tamaulipas 8
	Salvadoreño	Salvadoreño Amarillo
	Argentino-Criollo	Tequisate Golden Yellow
South America	Northern Cateto	Guyana Francesca III
	Cuban Yellow Dent	Bahia III BCO
		Dentado Amarill
	Argentino-Criollo-Tuson	Nariño 330-Peru 330
		DV 103
India	Caribbean-Tuxpeño-India-USA	Composite A1
		Multiple Cross 2
		Multiple Cross
		Synthetic A3B
		Synthetic A11
Other	Tuxpeño-Caribbean-USA	Tuxpantiqua
		Veracruz 181 × Antigua Gr.2
		Usatigua
		Florida Synthetic

of pollen development before the tassels were removed were considered. Sanford et al.[59] reported considerably more N accumulated in fertile tassels than in sterile tassels. After pollen shedding, there was no difference in the N content of fertile and sterile tassels. Sterile plants accumulated more N in ears and husks than did fertile plants due to the greater yield and number of ears produced by sterile plants. There were only slight differences between fertile and sterile plants in N content of leaves and stalks. They suggested that the comparatively fewer ears per plant produced by the fertile versions were due to competition for N between the ear primordia and the pollen.

b. Development and Evaluation of the Non-Detasseled Baby Corn Variety, Kasetsart 1

In the 1992 early rainy season, MS(1,2,6,7 × SW2)BC_5 and (CU88A(18 × 19) × SW2)BC_2 lines having 100% of male sterility, two ears per plant, and good agronomic characters were selected to

TABLE 9.5
Means for Fresh Ear Weight of Two Male-Sterile Varieties Compared with Suwan 2 (S)C7 and Chiangmai 90 by Using Non-Detasseling and Detasseling

Variety	Fresh Ear Weight				Unhusked Husked Ear Wt. Ratio	Ears per Plant (no.)	Relative to Suwan 2 (S)C7 (%): Fresh Ear Weight				Relative to Suwan 2 (S)C7 (%): Unhusked Husked Ear Wt. Ratio	Relative to Suwan 2 (S)C7 (%): Ears per Plant (no.)
	Unhusked (kg ha⁻¹)	Husked (kg ha⁻¹)	Standard (kg ha⁻¹)	Standard (%)			Unhusked	Husked	Standard	Standard		
					Non-detasseling							
MS(1,2,6,7 × SW2)BC_5	7,669	1,644	981	66	4.68	1.8	140	221	169	83	64	120
MS(CU88A(18 × 19) × SW2)BC_2	7,175	1,294	875	71	5.61	1.8	131	174	151	89	76	120
Chiangmai 90	4,931	1,025	675	65	4.79	1.5	90	138	116	81	65	100
Suwan 2(S)C7	3,606	619	438	74	5.84	1.1	66	83	75	93	79	73
					Detasseling							
MS(1,2,6,7 × SW2)BC_5	7,769	1,163	869	78	6.71	1.7	141	156	149	98	91	113
MS(CU88A(18 × 19) × SW2)BC_2	7,081	1,019	844	81	6.96	1.7	129	137	145	101	95	113
Chiangmai 90	6,638	1,044	700	79	6.48	2.0	121	140	120	99	88	133
Suwan 2(S)C7	5,494	744	581	80	7.36	1.5	100	100	100	100	100	100
Mean	6,094	1,063	706	70	5.46	1.5						
F-test	**	**	**	—	**	**						
C.V (%)	11.8	11.2	14.4	—	7.0	9.8						
LSD (0.05)	1,019	169	144	—	0.57	0.2						
LSD (0.01)	1,363	225	194	—	0.77	0.3						

Note: Tested at Suwan Farm in 1991 late rainy season.

form Male-Sterile Suwan 2 or MS-Suwan 2 by using bulk seeds. In the 1993 dry season, a maintainer was developed from the selected 11 S_1 lines of Suwan 2(S)C7. During 1993 to 1994, the female parent (MS-Suwan 2) and the maintainer were improved for baby corn in isolation blocks. In 1995, the National Corn and Sorghum Research Center released the non-detasseled baby corn variety, MS-Suwan 2, to farmers and processing plants in the name of Kasetsart 1.[14]

From results of evaluation at 12 experiment stations in the 1993 rainy season, on average, Kasetsart 1 had unhusked (5,781 kg ha^{-1}), husked (1,094 kg ha^{-1}), and standard (756 kg ha^{-1}) ear weights, number of standard ears (64%), the ratio of unhusked to husked ear weight (5.29), and ears per plant (2.24) higher than those of Suwan 2, and not different from Chiangmai 90 (Table 9.6). Furthermore, harvest date (44 days) and ear aspect and color of Kasetsart 1 were the same as Suwan 2 (Table 9.7). However, Kasetsart 1 had higher plant and ear height (187 and 106 cm) and fresh plant weight (31,450 kg ha^{-1}) than those of Suwan 2. Also, it was comparable to Suwan 2 for resistance to root lodging, downy mildew, and other leaf diseases. Consequently, Kasetsart 1, the non-detasseled variety, can reduce labor cost of baby corn production and eliminate time-consuming detasseling.

c. The Development of the Non-Detasseled Baby Corn Hybrids

Aekatasanawan et al.[60] evaluated topcross baby corn hybrids for male sterility using Kasetsart 1 topcrossed with 15 baby corn inbreds of Suwan 2(S)C7, 16 field corn inbreds of various germplasm sources (e.g., Suwan 1, second-cycle recovered lines of Ki21 and Ki27, etc.), and five TSC 1 DMR inbreds. They found that male-sterile topcrosses of baby corn, field corn, and sweet corn inbreds had 8 (53.33%), 6 (37.50%), and 3 (60.00%) varieties, respectively. Most male-sterile topcrosses of sweet corn inbreds had nonsignificant unhusked, husked, and good ear weights higher than those of Kasetsart 1. The results demonstrated that some male-sterile topcross hybrids can be used as the non-detasseled baby corn hybrid. They also have been developing the non-detasseled baby corn single crosses. And they found that Kasetsart 2, which was developed from Ki28cms crossed with KSei 14004 or [(*sh2* Syn 29 × KS 1) × Suwan 3(S)C4-F_4-S_8-24-2-2-4], gave higher unhusked, husked, and good ear weights and better quality. The National Corn and Sorghum Reseach Center released Kasetsart 2, the non-detasseled baby corn single cross, in 1999.

B. Future Use

Future use of baby corn breeding will be concentrated on single-cross hybrids to meet needs of farmers (e.g., high yield, resistance to root and stalk lodging, harvesting in a short period, etc.) and of factories (high uniformity and standard of raw materials, such as cob size, color, flavor, etc.). Thus, inbred and hybrid development will be selected for the desirable traits. The improvement or recycling of commercial or elite inbreds to correct some weak points will be emphasized for potential use as female parents for producing superior baby corn single crosses.

The required germplasm sources for future use in breeding should be more developed (i.e., genic male sterility or cytoplamic male sterility, silkless, etc.) for producing baby corn single-cross to reduce costs of production for the tasks of detasseling, husking, and removal of silks by hand.

Various types of molecular markers, such as restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats or microsatellites (SSR) will be exploited for helping breeders to select desirable genotypes resistant to important diseases and insects (for example, DMR, corn borer, etc.). To assess genetic diversity in germplasm of interest to breeders, molecular markers will be useful for identifying and classifying heterotic groups. However, heterosis in greater number of young ears per plant will be beneficial to baby corn breeders, because heterosis for fast ear development, as in field corn, results in cobs that are too large.

TABLE 9.6
Means for Fresh Ear Weight of Kasetsart 1 Compared with Suwan 2(S)C7 and Chiangmai 90

Variety	Fresh ear weight				Unhusked to Husked Ear Wt. (ratio)	Relative to Suwan 2 (S)C7 (%)				
	Unhusked (kg ha^{-1})	Husked (kg ha^{-1})	Standard (kg ha^{-1})	Standard (%)		Unhusked	Husked	Standard	Standard	Unhusked to Husked Ear Wt.
Kasetsart 1	5,781	1,094	756	64	5.29	125	147	150	108	84
Chiangmai 90	6,088	1,188	775	60	5.13	131	161	154	102	81
Suwan 2(S)C7	4,644	738	500	59	6.30	100	100	100	100	100

Note: Evaluated at 12 locations in the 1993 rainy season: Suwan Farm and 11 Research centers and experiment stations of Chiangmai, Chainat, Sri Samrong, Phitsanulok, Phra Phutthabat, Suphanburi, Sakol Nakhon, Kalasin, Khon Kaen, Roi Et and Songkhla.

TABLE 9.7
Means for Some Agronomic Traits of Kasetsart 1 Compared with Chiangmai 90 and Suwan 2(S)C7

Variety	First Harvest[a] (d)	Ears per Plant[a] (no.)	Ear[b]		Height[a] (cm)		Root Lodging[c] (1–5)	Foliar Disease[b] (1–5)	Plant Aspect[b] (1–5)	Downy Mildew[b] (%)	Fresh Plant[b] (kg ha^{-1})
			Aspect[c] (1–5)	Color[d]	Plant	Ear					
Kasetsart 1	44	2.24	2.5	YW	166	106	1.7	2.8	2.8	1	31,450
Chaiangmai 90	42	2.34	2.3	Y	186	117	2.2	4.0	4.0	4	25,906
Suwan 2(S)C7	44	1.91	2.5	YW	158	98	1.8	2.5	2.5	0	27,431

Note: Evaluated at Suwan Farm in the 1993 late rainy season and Chiangmai Field Crops Research Center in the 1993 rainy season.

[a] Data averaged from Suwan Farm and Chiangmai Field Crops Research Center, respectively.
[b] Data taken from Suwan Farm.
[c] 1 = best, 5 = poorest.
[d] Y = yellow, YW = yellow–white.

VI. EXTENT OF USE

Baby corn production was initiated in Taiwan. However, the success of baby corn production occurs in Thailand,[11] where it has been continuously developing for more than 30 years. Baby corn is used in two ways, fresh or processed consumption. Baby corn ears are popular as canned ears or with stir-fried vegetables in Chinese–American and European restaurants.[18] Recently, a market for fresh baby corn ears in trays has emerged in Europe, mainly for use as a decorative, crisp vegetable in salads. Another type of product is canned baby corn juices and drinks developed by Kulvadee et

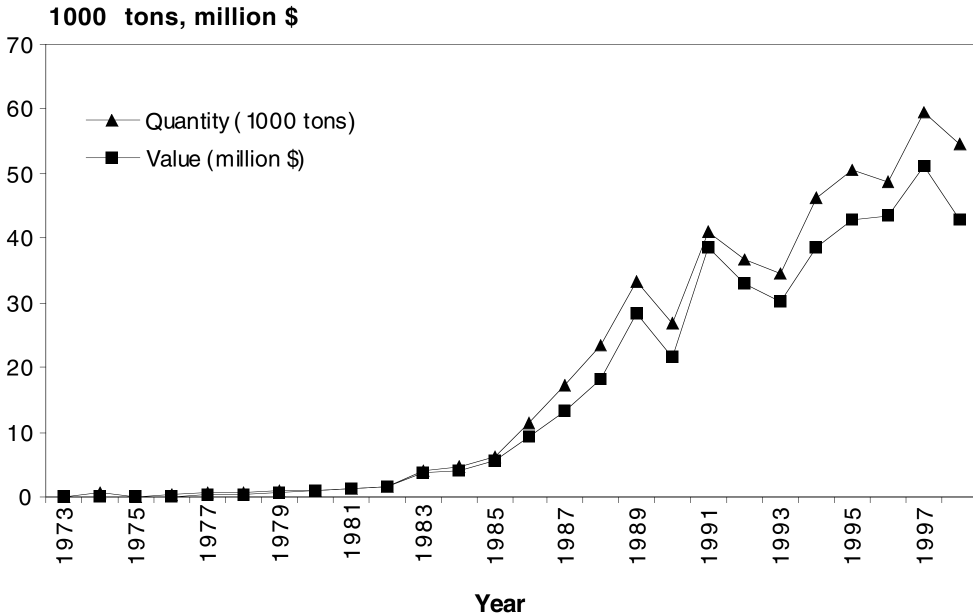


FIGURE 9.2 Quantity and value of Thai canned baby corn exports from 1973 to 1998. (*Source:* Thai Customs Department, Finance Ministry, Thailand.)

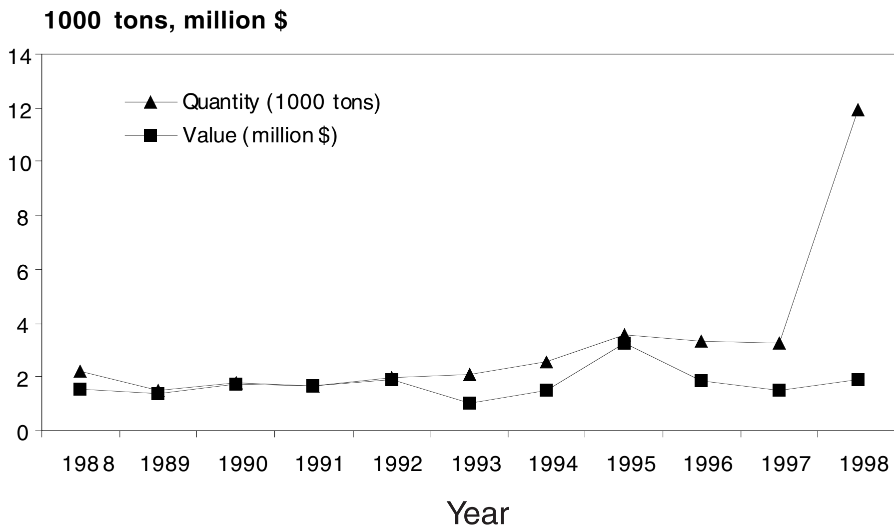


FIGURE 9.3 Quantity and value of Thai fresh baby corn exports, 1988 to 1998. (*Source:* Department of Business Economics, Ministry of Commerce, Thailand.)

al.[61] Baby corn is a popular vegetable because of its high nutritive value and freedom from pesticides compared with other vegetables. Generally, there is no need to apply pesticides: the young cob is wrapped tightly in its husk.

Thailand is the world's largest exporter of baby corn. In 1973, it exported only 90 tons of *canned* baby corn, worth $31,000. By 1998, the volume and value had increased to 54,643 tons, worth $42.89 million (Figure 9.2). Thailand also exported 2,220 tons of *fresh* baby corn in 1988, worth $1.54 million, but exports increased dramatically to 11,924 tons in 1998, worth $1.87 million (Figure 9.3). The greatest consumers of canned baby corn besides Thailand are the U.S., Netherlands, Japan, Germany, Canada, Australia, Hong Kong, U.K., France, Singapore, and South Korea, (Table 9.8).

TABLE 9.8
Exports of Canned Baby Corn from Thailand

	Quantity (tons)		
	1996	**1997**	**1998[a]**
U.S.	18,265	21,973	9,996
Netherlands	2,806	1,898	2,447
Japan	4,282	4,897	1,971
Germany	3,327	5,347	1,829
Canada	2,700	3,780	1,759
Australia	3,129	3,567	1,721
Hong Kong	1,606	2,568	1,535
U.K.	1,712	1,925	994
France	986	1,252	588
Singapore	1,346	1,578	401
South Korea	1,193	1,994	269
Other countries	7,286	8,806	4,762
Total	48,638	59,585	28,272

[a] January to June.

Source: USDA/FAS.

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10 Blue Corn

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I. INTRODUCTION

Blue corn has traditionally been a flour corn, *Zea mays amylacea*. It is a soft corn with long ears. Generally it has 8 to 12 rows of grain with a soft, floury endosperm without dents or wrinkles. All colors may be present.[1]

The center of origin of floury corn is in the Andes, primarily in Peru, where floury corns are popular. The Cuzco corn is famous as the origin of Cornnuts. The large, floury kernels grown in that region are preferred for special snacks in Japan. Floury corn is not widely grown in Mexico, but it is abundant in limited areas of the central tablelands, where it is preferred for tortillas and atoles. The popularity of floury corns may be due to their relative easy reduction into flour or reduced cooking times in alkali. Thus floury corns became popular in hot, dry regions where the grain did not deteriorate and presumably insect damage was minimal.[2,3] In contrast, the soft floury corns were damaged by insects in hot, humid areas; kernels planted in cold soils and humid tropical areas molded and failed to emerge from the soil. It is well known that the soft opaque high lysine corn kernels were preferred by insects during storage, and stand establishment was difficult. On the other hand, the hard, flint corns could be grown more successfully and became the variety of choice, though processing into foods required greater energy. Thus, dent and flint corns predominate in production.

II. HISTORY

Corn has been associated with many great cultures in the New World, including those of the Inca, Maya, and Aztec civilizations. Most notably, the modern day North American Indian tribes —

Zuni, Hopi, and Navajo — still prefer flour corn of various colors. Blue corn is especially prized as a ceremonial corn.[3,4]

The various Indian tribes were aware that the different types of corn would cross-pollinate if they were either interplanted or adjacent to each other. Thus the Indians established strict guidelines as to the isolation required to prevent cross-pollination so they could maintain purity of the various types.[5]

Southwestern American Indian tribes utilize blue corn as a food source as well. It is normally dried, stored on the cobs, and ground into meal as demanded. The cuisine of New Mexico is significantly related to these native preferences; therefore, blue corn production and products originated in that area. It has spread to other U.S. areas as special products and served in upscale Mexican restaurants as organic tortilla chips and other foods.

III. PLANT AND KERNEL CHARACTERISTICS

Most commercial hybrid dent corns are uniform and produce grain yields of 8 to 13 tons per hectare. The open-pollinated blue corn varieties produce significantly reduced grain yields, thus, the cost of blue corn is significantly more than dent corn. Blue corn varieties are highly variable for plant and kernel characteristics. They are late with longer flowering periods, variable maturation times, and produce a greater number of tillers. Blue corn varieties have poor stalk strength and often lodge prior to harvest. High winds break off the stalks and cause significant losses of grain and lower the quality of the kernels. Mechanically harvesting the crop is often difficult because of the lodging.[6] The varieties are very susceptible to diseases and insects when they are grown in different environments from those where they originated.

Blue corn production is similar to that of dent corn in general. However, it does not respond to intensive management practices used for dent corns because of its lack of adaptation. It does well when grown using organic farming techniques or moderate levels of fertilizers and other management practices. Its long and variable maturation time requires some care in overcoming insects and diseases. Some blue corn hybrids that have improved yields, plant height, and maturity have been developed. These hybrids often have only marginal blue color in the finished products.

The corn kernel is composed of pericarp, germ, and endosperm which consists of a single layer of aleurone cells and corneous, floury, and peripheral endosperm. The relative proportion of floury to corneous or flinty endosperm varies from all flinty to nearly all floury endosperm. The floury endosperm consists of a discontinuous protein matrix with large spherical starch granules and empty spaces between starch granules, protein bodies, and matrix. These voids refract light rays and appear light to reflected light or opaque to transmitted light. The aleurone layer contains the blue anthocyanin pigments that give the blue color. Most blue corns grown in the Southwest and Mexico are floury. The outer layer is the pericarp that consists of several layers of cells that protect the kernel. In addition, remnants of crushed cells, called the testa or seed coat, can be seen with a microscope.

Kernel size, shape, and structure vary significantly. The kernels of small to medium size produce the darkest blue color products because the smaller kernels have a higher proportion of aleurone layer and are diluted less by the endosperm. Pigmentation can occur in the pericarp, aleurone, and starchy endosperm; however, the blue color is located in the aleurone layer of typical blue corns. Sometimes the pigmentation is so intense that kernels appear to be black. Yellow endosperm color is highly undesirable because it causes a greenish yellow off-color.

IV. HARVESTING, STORAGE, AND MARKETING

Mechanical harvesting, handling, and storage of blue corn are critically important, because the soft, fragile kernels are easily cracked, broken, or fissured, which significantly reduces quality of the grain for most end uses. Rotary combines are preferred, as they reduce the percentage of cracked kernels. Blue corn is often harvested at approximately 18 to 20% moisture followed by drying with

relatively low-temperature hot air dryers to around 13 to 14% moisture for safe storage. Gradual reduction in moisture content reduces the fissuring and improves quality for alkaline cooking. Rapid drying causes stress cracks and fissures. Harvesting at lower moisture levels increases the percentage of cracked kernels significantly. Blue corn with a higher percentage of cracked kernels has significant dry matter losses during alkaline cooking especially in automated tortillerias.[6] For dry milling into flour and meal, fissuring is of less importance, since the whole kernel is ground anyway.

Storage of blue corn is similar to dent corn storage except handling causes significantly greater breakage. Mechanically handling blue corn often causes a higher percentage of broken kernels. Food corn suppliers and processors must work together to maintain its quality. Blue corn can contain aflatoxins and other mycotoxins, so they should be monitored.

Consumption of blue corn products is increasing. Most blue corn is grown under contract with specifications depending on its ultimate end-user or product. Common sense is required to establish realistic standards that both buyer and seller can tolerate over a long-term relationship. For example, the floury corns have lower test weight and density, so that must be considered in establishing standards. In general, the grain should be cleaned to remove broken, cracked kernels, foreign material, and other impurities. The amount of nonblue kernels must be limited so corn should be grown under isolation; 2% or less of nonblue kernels is realistic. Specifications for damaged kernels determined according to Federal Grain Inspection Service standards for dent corn are reasonable. Chemical residues, aflatoxins, and other mycotoxins must be monitored and kept within acceptable levels. The moisture content is usually around 13% moisture with no more than 10% of stress cracks.[6] For safe storage, the moisture content should be 12 to 14% depending upon expected length of storage, the type of storage container, and the management.

Corn with an intense dark blue color is critically important. It is usually associated with a soft floury kernel of modest size, so kernel weight is important. Definite standards are not available for blue corns; buyers develop their own or accept corn that is available. They have variable quality, color intensity, and flavor. The most intense blue colors are found in corn from New Mexico and selected areas of Mexico. Organic blue corns can be certified in states with regulations.

V. BLUE CORN PRODUCTS

A. Nixtamalized Products

Tortillas, tortilla chips, baked tortilla chips, posole, atole, and other products are made from blue corn by the alkaline cooking procedure described (Chapter 11). The blue corn is made into nixtamal by treating dried kernels with lime and water to remove the pericarp (hulls). The nixtamal may be dried, stored, and used in stews, soups, and other products (posole). The washed nixtamal is stone ground to make masa for tortillas, tortilla chips, atoles, and tamales. The dark blue color affects the taste and acceptability of the products; they are considered more flavorful than white corn products.

Because of its soft endosperm it is difficult to cook blue corn to achieve desirable masa properties while retaining the desired blue color intensity. Cooking time, temperatures, level of lime, extent of washing, and pH affect the yields and color of the products significantly. The darkest color is obtained at pH of 8.0. Violet colors are prevalent at lower pH while higher pH gives a greenish color.

B. Meals, Flour, and Products

Blue corn is crushed using stone mills and sifting to remove some of the very coarse pericarp to produce refined meal and flour depending upon the particle size desired. These ground essentially whole grain products are used to produce a number of foods. In the southwestern U.S., pika or paper bread is produced by Navajos especially for special ceremonial occasions. The fine corn

meal or flour is made into a thin batter; boiling water is added to form a thin paste, which is spread on a hot, flat stone or metal griddle that has been brushed with vegetable oils. The thin layer after baking is peeled off and folded or rolled into thin wafers. The pika is generally eaten with beans or stew.[7] Fussel[8] speculated that pika was the original precursor to our modern corn flakes.

Blue corn meal or flour is made into a thick porridge called Chaqueque in the southwestern U.S. It is similar to corn meal mush or gruel, which was a common food of early Americans, where only yellow corn was used. The corn kernels are toasted before being ground, giving the porridge a significantly different flavor and aroma.

Atole de maiz is a thin porridge that can be drunk, since it has a consistency like thick cream. It is usually made from alkaline cooked masa, but in some cases flour or meal obtained by dry milling was used to produce the product. Aztecs and Mayans often added other ingredients to improve flavor and vary the product taste and texture. Each *atole* had a different name, depending on its ingredients. Blue corns produce a special *atole*.

Pinole is usually a beverage made from a mixture of ground, toasted blue corn blended with cinnamon, sugar, honey, other special seeds, and ingredients. This lightweight, highly nutritious food is used directly or mixed with water or milk to drink. Sugar, honey, or other sweeteners are added to *pinole* to improve its palatability. *Pinole* is also made from white and yellow corns.

Chicos are made from immature corn kernels steamed in the husk and dried.[6] *Chicos* are generally cooked with beans, chile, and green onions, or used in stews. The kernels were roasted to inactivate enzymes, and a unique smoky flavor was imparted to the *chicos* which enhanced their flavor. Commercial products are available in specialty stores in New Mexico.

Chicha morada is a fermented alcoholic beverage made from soft, purple or deep blue corn in Peru and Bolivia. *Chicha* is a corn beer made from malted soft corn which is ground, mashed, filtered, fermented, and flavored with various additives. *Chicha*, which is not always alcoholic, is a very significant product, because it does have higher economic value over corn. The deep purple *chicha morada* is especially popular on ceremonial occasions.

Many new snacks, breakfast foods, and other products from blue corn are available in health food stores and supermarkets. These products include pancakes, muffins, corn flakes, a wide variety of extruded snacks, and other breakfast foods including blue corn flakes. The flavor and color make the products attractive with a unique appeal to some customers. Several companies produce baked, low fat, blue corn tortilla chips. Some contain other components like sesame seed and related products. In many instances the products are made with organically grown blue corn and command a premium price. Novelty products like natural red, white and blue corn tortilla chips are sold. Blue corn is extruded, popped, flaked, and made into a variety of products. Expansion of the blue corn industry depends on promotion of both traditional products and new products. In addition, a source of less expensive intense blue corn is required.

VI. BREEDING BLUE CORN

A. Genetics of Blue Color

Blue color in corn is due to anthocyanins in the aleurone layer. Several factors control the color expression in the aleurone layer: anthocyaninless-1 (*a1*), anthocyaninless-2 (*a2*), bronze-1 (*bz1*), bronze-2 (*bz2*), colorless-1 (*c1*), colorless-2 (*c2*), defective kernel-1 (*dek1*), red aleurone (*pr*), colorless (*r*), and viviparous-1 (*vp1*). Deep purple color requires the presence of a dominant allele at every factor: *A1, A2, Bz1, Bz2, C1, C2, Dek1, Pr, R,* and *Vp1*.[9] The blue pigmentation is intensified if factor *in* is homozygous recessive (Table 10.1). Brownish coloration is developed if *bz1* or *bz2* is homozygous. Factor *c1* has different alleles: *C1-I* (inhibitor), *C1* (color-determining), and *c1* (colorless). These alleles show dosage effects. Genotypes *C1/C1/c1* are purple while *C1/c1/c1* are pale unless *in* is present. Genotypes with *C1-I* are colorless. However, the allele *C1-S* for strong coloration gives blue color even in the presence of *C1-I*. Recessive factor *c2* develops blue color

TABLE 10.1
Combination of Alleles that Yield Blue Color Aleurone Under the Modifying Factor *In* in the Presence of at Least One Dominant Allele in All the Other Factors

Factor	*In_ _*	*in in in*
All color factors present	Purple	Deep purple; pericarp brown
bz1 bz1 bz1	Purple bronze	Brownish purple; pericarp brown
bz2 bz2 bz2	Purple bronze	Brownish purple; pericarp brown
C1/c1/c1	Pale purple	Purple
C1-I/C1-S/C1-S	Pale purple	Purple
c2/c2/c2	Pale purple	Purple
C2-Idf/C2/C2	Pale purple	Purple
R/R/r	Purple	Deep purple
R/r/r	Mottled purple	Mottled deep purple

Source: Adapted from Coe, E. H., Neuffer, M. G., and Hoisington, D. A., in *Corn and Corn Improvement*, 3rd ed., Sprague, G. F. and Dudley, J. W., Eds., American Society of Agronomy, Madison, WI, 1988.

in the presence of factor *in*. Blue color can be reduced by the presence of dilution factors such as *C2-Idf*. The expression for the *R* locus alleles (*R* and *r*) shows dosage dependence. While genotypes *R/R/r* are purple, genotypes *R/r/r* are mottled purple.

B. Germplasm Sources for Blue Corn

Germplasm sources for blue corn can be found in several countries of Central and South America (Mexico, Peru, Bolivia, Guatemala, etc.). Blue grain accessions exist at the CIMMYT (International Center for Wheat and Maize Improvement) and Latin American gene banks. In the U.S., blue corn varieties are found in the Southwest (New Mexico, Colorado, Arizona). The identification and characteristics of blue corn accessions found in the online database of GRIN (Germplasm Resources Information Network),[10] available from the U.S. Department of Agriculture/Agricultural Research Service National Plant Germplasm System, are presented in Table 10.2.

TABLE 10.2
Blue Corn Accessions and Their Characteristics

Accession	Characteristics
Hopi Blue	Improved cultivar.
Crumpacker47	Hopi Indian blue corn landrace.
Ames 14263	Blue grain collected in Georgia.
Ames 22778	Blue Fox Flour Corn: Kickapoo Indian cultivar.
NSL 26565	A blue aleurone selection of OSU 106 (Oklahoma).
PI 213738	Blue Navajo. White cob.
PI 213768	Great plains blue flour-corn landrace.
PI 278708	Blue Clarage. Blue aleurone, adapted to Central Ohio.
PI 317683	Blue Ridge White Cap. Red cob, susceptible to cornborer.
PI 420246	Pueblo race. Blue flint-flour type.
PI 476866	Grown by Warihio Indians. 12 rows, slightly dent blue seeds.
PI 476867	From Pueblo Indians on irrigated flood plain of Rio Grande. Floury.
PI 476869	Drought tolerant, cobs 12 to14 rows, floury.
PI50356	Pueblo preliminary race. Floury with average of 12.5 rows.

Most of the blue corn is open-pollinated varieties (OPV). These OPVs are adapted to dryland conditions and low input management techniques. Current cultivars are susceptible to European corn borer (*Ostrinia nubilalis* Hubner). Western corn rootworms (*Diabrotica virgifera*) can affect blue corn yields on soils under continuous corn production. Selections derived from blue OPVs have shown poor performance due to low yields and susceptibility to ear rot, root lodging, and stalk lodging. Evaluations under optimal conditions of fertility and water increase plant height which accentuate lodging.

The effort in genetic improvement of these populations has been minimal. However, the increased consumption of blue corn in high value foods is promoting the development of commercial hybrids. In addition, these populations could represent a unique source of alleles for important economic traits in corn such as drought tolerance and nutritional value.

Johnson and Jha[11] characterized five Hopi blue flour corn populations from New Mexico and Colorado. Tillering was variable ranging from 2 to 10 tillers per plant. Kernel thickness was correlated with cob diameter, and ear length was correlated with grain yield in all populations. They indicated that weight, width, and thickness of the kernel are the most relevant components of yield for these races of blue corn. The ear characteristics in Hopi blue corn are singular particularly for ear width and shank diameter. Soleri and Smith[12] compared two Hopi corn varieties conserved *in situ* and *ex situ* for morphological and phenological traits. They found significant differences between the blue corn population maintained *ex situ*, which apparently suffered genetic shift and genetic drift, compared with that maintained *in situ*.

C. Conversion of Elite Corn Inbreds to Blue Corn

The conversion of elite field corn lines to blue corn is the breeding approach used to overcome the agronomic deficiencies of the traditional blue corn varieties. White-grained inbreds are preferable because it facilitates recovery of the deep blue color. At Texas A&M we are converting white inbred lines to blue color following a modified backcross breeding approach of alternating selfing and backcrossing (Figure 10.1). The donor for blue color (DBC) is crossed with the white inbred line (WIL) to convert recurrent parent. The resulting F1 cross is selfed. The purpose of selfing is to fix the dominant alleles in regulating factors. The F2 ears will segregate for white and blue kernels. The most desirable deep F2 blue kernels are selected. It is important to screen enough F2 ears to increase the probability of obtaining genotypes carrying dominant alleles in most of the factors. F2 plants from these kernels are used as females in the cross (BC1) with the white recurrent parent. The procedure is similar for subsequent backcrosses (BC2, BC3, etc.). Because endosperm dosages affect some of the genes or modifiers that regulate the blue color, the blue corn should be used as females. Finally, converted lines can be evaluated in hybrids for grain yield, agronomic performance, quality traits, processing, and nutritional value. Conversion of inbreds from different heterotic groups and genetic backgrounds should lead to good performing blue hybrids.

D. Hybrid Evaluation

Several commercial hybrid blue corns have been developed and are beginning to be grown, which will increase the yields, reduce cost, and improve the availability of blue corns for processing. These hybrids have a harder endosperm and can be more easily processed with the standard equipment presently being used in the industry. Unfortunately, the color of the products is usually less than that desired but progress is occurring.

Grain of six experimental blue corn hybrids and a standard soft blue corn was processed into tortillas and tortilla chips in our laboratory using pilot plant processes (Table 10.3). The blue corns also were evaluated objectively and subjectively at different stages of processing for color and acceptability (Figure 10.2). The standard blue corn was softer and had significantly lower density compared with the experimental blue corns which allowed it to reach optimum cooking time more

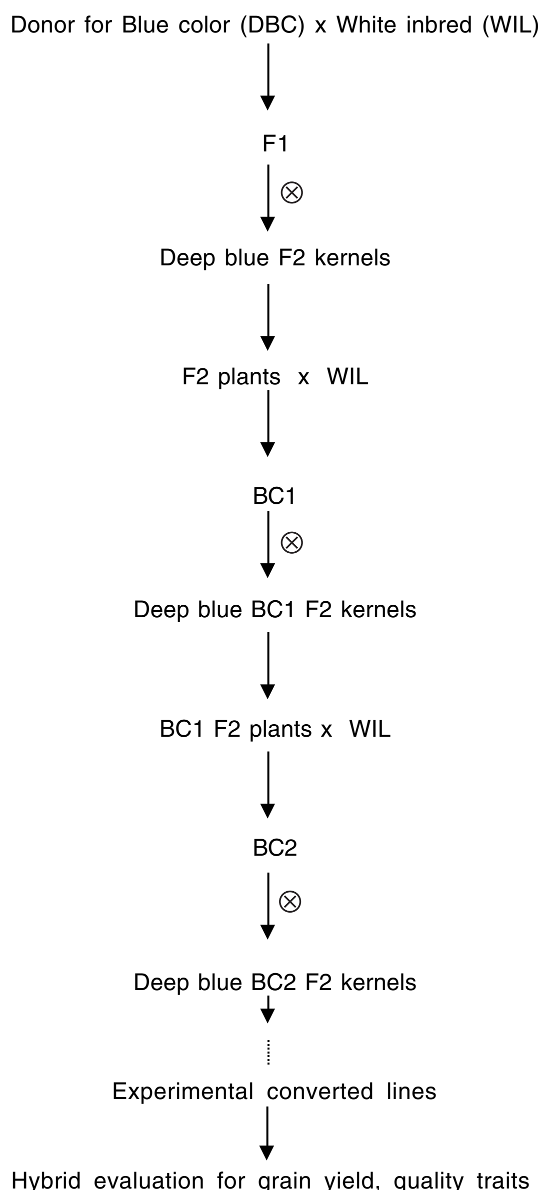


FIGURE 10.1 Breeding scheme at Texas A&M University to convert white inbreds to blue color.

quickly (Table 10.3). The harder corns required significantly more time for cooking with greater dry matter losses.

The standard blue corn produced an acceptable blue color in tortillas and tortilla chips (Figure 10.2). Among the experimental blue corns, corn No. 2 had the darkest blue color of all the blue corns in both tortillas and tortilla chips. The blue color decreased significantly during processing for all of the blue corns including the control. However, corn No. 2 had colored products equal to the control. The blue corn No. 5 had a very low color that was unacceptable. The tortillas and tortilla chips from corn No. 5 were a greenish-blue color, because it had a yellow endosperm. The other four blue corn hybrids did not produce acceptable colored products.

TABLE 10.3
Physical Processing Properties of Hybrid Blue Corns Compared with Open Pollinated Blue Corn

Blue corn Samples	Test Weight (lb/bu)	TKW[a] (g)	Density (g/c^3)	Hardness (% removed)	Floaters (%)	Optimum Cooking Time[b] (min)	Dry Matter Loss at Optimum Cooking Time[c]
Standard	56.7	293	1.23	52	100	–23	3.7
1	63.1	375	1.32	43	41	28	8.2
2	61.5	367	1.28	45	76	14	7.9
3	63.1	425	1.32	41	14	31	8.8
4	63.7	364	1.33	37	25	31	8.7
5	63.3	307	1.34	45	19	11	9.6
6	62.0	280	1.31	42	72	11	9.1

[a] Thousand kernel weight.
[b] 0 minutes of optimum cooking time is 3.5 hr and 23 min means that the corn was added 23 min after the corn-lime had reached 0 time and the steam was turned off.
[c] % of initial dry weight of sample.

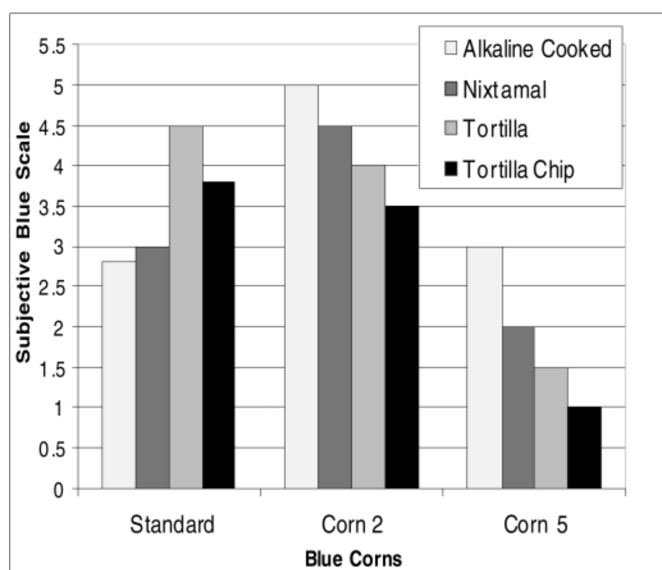


FIGURE 10.2 The effect of processing steps on the color of blue corns. Alkaline cooked is after cooking while nixtamal was steeped and washed. 1 = light blue, 5 = intense blue.

Although the hybrids had better physical properties than the standard blue corn, the color of the tortillas and tortilla chips for 5 of the 6 hybrids were lighter and did not give a desirable blue color. The fact that No. 2 had products with color similar to the control floury blue corn is encouraging. This shows that harder, higher yielding blue corns equal to soft blue corn in color are being developed.

During processing, the experimental hybrids lost some of the blue color. Additionally, pigments in the endosperm affected the final color of the tortillas. The yellow pigments in the endosperm of some of the experimental blue corns contributed to the greenish color of the tortillas. This problem can be circumvented by converting white endosperm inbreds rather than yellow endosperm inbreds.[13] Blue corns can be improved in terms of yield but the use of soft, white endosperm inbreds appears helpful. The soft endosperm may be desirable for some processes.

Over time blue corns with greater yields and desirable processing and color will occur. Then, the unique flavor and color will be used in a greater array of products.

VII. NUTRITIONAL VALUE

The nutritional value of blue corns with the floury endosperm is higher than yellow or white dent corn grains. Dickerson[6] reported that the lysine content of five blue corns (floury) was 2.3 mg/g protein compared with 1.4 for two samples of hybrid dent yellow corn. In general, the protein and mineral content of blue corn are higher than that of most dent corns. The blue corn contains higher levels of flavanoids especially anthocyanins, which are currently thought to be excellent sources of antioxidants for functional foods. Thus, blue corn has some nutritional advantages but its major allure is its blue color and different taste.

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11 Food Uses of Regular and Specialty Corns and Their Dry-Milled Fractions

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I. INTRODUCTION

Corn or maize (*Zea mays* L.) is the third largest grain crop in the world and the leading cereal in the U.S.[1] This cereal is a staple for large groups of people in Latin America, Asia, and Africa, where it is used for the production of numerous traditional foods. Corn foods are characterized for their unique distinctive flavor not duplicated by any other cereal. Food products from corn are found in almost everything we eat. In the U.S., where 247.9 million metric tonnes were produced in 1998, the grain is generally used as animal feed and for production of corn meal, flour, grits, starches, sweeteners, cooking oil, industrial alcohol, alcoholic beverages, tortillas, snacks, breakfast foods, and other products. The use of corn products as an ingredient in ready-to-eat breakfast cereals and mixes has increased as the American consumer looks for convenience foods that meet nutritional and health requirements.[2] The alkaline cooking process of whole corn has become important in the U.S. due to the increase in popularity of Mexican foods. In 1998, sales of U.S. made corn and tortilla chips totaled $0.74 and $3.57 billion.[3]

The dry milling industry and food processors are demanding corns with improved characteristics so better yields and quality products can be obtained. Development of these corns can also benefit the expanding U.S. export market. Some unique types such as blue corn are becoming increasingly popular in specialty food stores. Other corns, such as the improved high lysine, quality protein maize, could help the animal feed industry by decreasing feed costs or by decreasing protein malnutrition in underdeveloped countries where corn is a staple food. Recent references of general interest on food use of corn are available.[4–18]

Food and industrial uses of starches, sweet corn, and popcorn are discussed in previous sections. This chapter summarizes the use and selection of regular and speciality corns for traditional and industrial foods with special emphasis on alkaline cooked corn products (tortillas and snacks), other snacks, and breakfast foods.

USES OF CORN

World corn production in 1998 was estimated at 604 million metric tonnes. Approximately 40% was harvested in the U.S.[1] Other important producers include China (125.4 million tonnes), Brazil (29.3 million tonnes), Argentina (19.1 million tonnes), Mexico (18.4 million tonnes), France (14.4 million tonnes), and Rumania (8.6 million tonnes). In the U.S., 80% of the corn is fed to livestock. The rest (more than 40 million tonnes/year) is processed into food and industrial products. Most of the corn produced in developing countries is processed into indigenous foods. In Latin America, corn is generally processed into tortillas, arepas, couscous, polenta, and various meals that are the base for many traditional foods.[18] Per capita supply of corn in Mexico is 128.6 kg/year. Twelve million tonnes are used directly as food and one million tonnes are industrially processed.[1]

In Africa and Asia, corn is generally dry-milled into grits or meals and flours for production of flat breads, i.e., roti, corn bread, unfermented and fermented porridges (Ogi, Tô, Ugali, Pap), steamed foods (couscous, rice-like corn grits), snacks (popped corn), and alcoholic or non alcoholic beverages.[9]

III. TRADITIONAL CORN FOODS

Corn is widely consumed by humans in Asia, Africa, Latin America, and the Balkans. White maize is generally preferred for food uses, although yellow maize is also utilized in many products and exclusively in some areas. For example, Brazil prefers yellow maize.

A. Milling

Most traditional corn foods are produced from grain that is milled into a meal or flour. Traditional milling is based on the use of stone mills and wooden mortar and pestles. These milling procedures are still widely used in Africa, Latin America, and Asia.

In pioneer America, corn was milled into whole meals with the use of Indian metates and manos, European mortars and pestles, and the widely employed home grinder called hominy block.[19] Milling evolved with the introduction of the quern, a small, stone burred grinder apparently invented in ancient Rome. Querns consisted of a pair of stones: one static, called a bedder, and the other rotating cap stone with a hole, called a tedder. Corn kernels were ground into a coarse meal. These revolving stone mills soon replaced the metates and were applied on a commercial scale. Energy to operate the mill was supplied by livestock, humans, wind towers, and more often by water.[19] Whole ground corn meal became rancid rapidly. Thus, many small mills produced corn meal for local villages and towns. The invention of degerminators in the early 1900s allowed for the production of low fat meal with good shelf stability that permitted large-scale milling. Today, most corn is degerminated during dry milling.

B. Traditional Foods

Corn is consumed in numerous forms. The use of fresh or immature corn on the cob is practiced. Corn cobs with or without husks are boiled in water or cooked over a fire and then flavored with salt, cream, butter, margarine, and other sauces. In most areas, field corn is used as green corn. Sweet corn is widely used only in the U.S. Tortillas and arepas are the most important corn foods for people in Mexico, Central America, Venezuela, and Colombia (Table 11.1). In Mexico, nearly 52% of the corn is used for human food, mainly in the form of nixtamalized (lime-cooked) products. In particular, the lower socioeconomic groups depend on tortillas as the main source of calories and protein. Paredes-Lopez and Saharopulos-Paredes[39] estimated that the average annual per capita consumption of corn in Mexico, mainly in the form of tortillas, is 120 kg. Recent data clearly shows that the dry masa flour industry processes 3 million tonnes/year with a capacity of more than 5 million tonnes of tortillas. These industries produce approximately 40% of the total tortillas produced in Mexico; the rest is produced from nixtamal or fresh masa.[40] Krause,[41] likewise, found that Guatemalan Indian women consumed 579 g of tortilla per day, which contributed 33 g of protein, 642 mg of calcium, and 1.164 kcal of energy.

The traditional method to process corn into tortillas, called nixtamalization, was developed by ancient Mesoamericans. In nixtamalization, water containing lime is used to cook the grain; although the leachate of wood ashes is still used in some villages in Mexico and Central America.[17] The lime-cooked corn, called nixtamal, is washed by hand to remove excess lime and pericarp tissue, and ground on a metate or stone grinder to form a dough called masa. Masa is the base for traditional products such as tamales, pozol, atoles, tortillas, tortilla chips, and other products. For tortilla production, small portions of masa are hand-shaped into flat discs that are baked on a clay comal or hot griddle for 30 to 60 s on each side.[17]

The traditional tortilla-making process involves cooking corn with lime in a pot over a fire for 5 to 50 min and steeping for 8 to 16 h (Figure 11.1). This is the method by which over 90% of the tortillas consumed in Guatemala are prepared.[41] In Mexico, a table tortilla is thin and puffs during baking, whereas in Central America most tortillas are thick and may not puff during baking. Combinations of beans, various meats, cheeses, and vegetables generally accompany tortillas. Serna-Saldivar et al.[18] listed the most important traditional masa/tortilla-based dishes of Latin America. The description of traditional fermented masa products is discussed by Steinkraus[9] and is summarized in Table 11.1.

Arepas, the national corn bread of Venezuela and Colombia, are traditionally produced from white corn grits or meal that is moistened, cooked in water, and ground to a dough. The dough is hand-shaped into flat disks approximately 7.5 cm in diameter and 1 cm thick. The disks are browned on each side and baked in an oven. Sometimes, gas-fired grills with open flames are used to impart characteristic toast marks. Arepas are cut in half and stuffed with meat, cheese, butter, jellies, and other fillings. Stuffed arepas are sometimes fried to produce hallaquitas, hallacas, empanadas, and other foods. Unfried arepas contain 58 to 64% moisture, 4% protein, 0.7% fat, 38% carbohydrates,

TABLE 11.1
Traditional Foods Made with Corn

Food	Common Name	Country	Process
Whole grain			
	Hominy	U.S.	Corn is cooked in lye to soften the grain and increase palatability.
	Pozole	Mexico	Lime-cooked or lye-cooked corn kernels are prepared into a spicy (pepper mainly) soup that contains pork or other meats. Menudo is similar but prepared from beef stomach.
	Nixtamal	Mexico, Central America	Corn is cooked in lime or ashes, steeped for 8–16 h and washed with water to remove pericarp. Nixtamal can be used for preparation of soup or in most cases is stone-ground into a dough or masa, which is the basic product for the production of numerous nixtamalized foods (tortillas, chips, tamales, atoles, beverages, etc.)
	Munguçá	Brazil	Degerminated-dehulled white corn is cooked in coconut milk with clover and cinnamon.
Thin porridges			
Unfermented			
	Atole	Mexico, Central America	Raw corn is ground into flour or cooked in lime or water to produce masa. The dry flour or wet masa is wet-sieved to remove coarse particles. The slurry is boiled for 15–20 min and blended with milk, sugar, cinnamon, and other flavorings. Atoles from immature kernels are also produced.
	Pinole	Mexico	Same as atole but kernels are first roasted on a griddle for 3–15 min. The product has a granular texture and stronger flavor.
	Chicha Morada	South America	Blue corn is cooked in water with sugar for several hours. The mixture is filtered and the purple liquor is blended with fruit juice (i.e., pineapple) and consumed as a refreshing beverage.
	Mingau	Brazil	Corn grits are mashed immature kernels are cooked in water to produce porridges similar to atole.
	Canjica	Brazil	Canjica is degermed corn kernels cooked with sugar and milk, and generally consumed as a dessert or as a breakfast cereal.
	Pamonha	Brazil	Pamonha is produced from a slurry of immature corn that is steam-cooked in a sac of corn husks.
Fermented			
	Ogi	Nigeria	Corn kernels are steeped and fermented for 1–3 days. The grain is wet milled, slurried, and sieved with water. The slurry is allowed to further ferment for 1–2 days. The fermented sediment is separated and boiled in water to yield ogi porridge that is consumed warm or cooled to form a gel or pudding.
	Uji	Africa	Corn is finely ground and slurried with water (30% w/v). The slurry is allowed to ferment naturally for 2–5 days until 0.3–0.5% acid is developed. The slurry is diluted with water (8–10% solids), boiled, and flavored with sugar. The Uji can be consumed warm as a creamy soup, or in the form of a thick paste called Ugali.
	Mahewu	South Africa	Corn meal is mixed with water (8–10% solids), boiled for 1.5 h, cooled, and blended with semolina or flour (5% of the corn weight). Wheat serves as an inoculum for fermentation. The blend is incubated in a warm place for 36 h to develop the desired sour flavor.

Thick porridge			
	Hanchi	South America	Corn grits are cooked in water containing sugar, dried peach, and prunes for 1 h. Then, upon cooling, lemon juice is added. Hanchi is a yellow, sweet and pudding-like product.
	Mazamorra	South America	White corn is soaked overnight and cooked in water and/or milk for 2 h. The cooked kernels look like hominy, but they are cooked without alkali and they are not washed. Sugar is also added during cooking to enhance the flavor. The product is consumed after cooling.
	Maizena	Mexico	Corn starch and sugar are cooked in water and/or milk for a few minutes until starch is gelatinized. Maizena is flavored with orange leaves, vanilla, or cocoa. Commercial maizena blends are available.
	Humita	South America	Fresh corn is separated from the cob and ground. The ground corn is mixed with butter, salt and spices. Small portions of the resulting dough are spread onto fresh husks and then filled with cheese or meats. The humita is wrapped in fresh corn husks and cooked in water for 20–40 min.
	Tô, Tuwo, Asida	Africa	Cornmeal is cooked in water until complete gelatinization. Additional corn flour is added and stirred until a very stiff paste is formed. The porridge is placed in a gourd, cooled for 1 h, and eaten with the fingers and a sauce. Sometimes acid (tamarind, lemon) or alkali (wood ashes) is mixed with the cooking water to produce acid and alkali Tô. Granulation of the flour, composition, type of sauce, and final consistency of the product vary among countries and tribes within a country.
	Polenta	South America, Europe	Degerminated corn grits are cooked in water until complete gelatinization. The cooked meal is either mixed with tomato sauce and cheese and baked or blended with tomato sauce containing ground meat.
	Finger bread	Southwestern U.S.	Blue corn meal is boiled thick with water and served on a plate. Bite-sized pieces are consumed with condiments.
Snack foods			
	Popcorn	Worldwide	Special flint corns with vitreous endosperm are popped in oil, hot air, or on a hot surface. The popcorn is generally salted or flavored with cheese or spices.
	Corn on the cob	Worldwide	Sweet (U.S.) or regular corn cobs (immature; dough stage) are dehusked and boiled, broiled, or barbecued. The corn on the cob is generally salted and flavored with margarine, butter, mayonnaise, or spicy condiments.
	Corn and tortilla chips	Central and North America	Lime-cooked corn is stone ground into masa. Corn chips are produced by frying pieces of masa. Tortilla chips are produced from pieces of masa that were baked before frying.
Steamed foods			
	Couscous, Cuzcuz	Africa, Brazil	Finely ground corn is kneaded with water until the flour particles agglomerate. The mixture is sieved through a coarse mesh. Then the particles are steam cooked in a container with a perforated bottom. During cooking, the couscous is removed, resieved, and returned to the steamer for further cooking. Usually ground baobab leaves, peanut butter, okra, or some other flavorings are mixed with the couscous during the final stage of steaming. The cooked product is consumed with a sauce. Sometimes, it is dried and used as a convenience food.

continued

TABLE 11.1 (CONTINUED)
Traditional Foods Made with Corn

Food	Common Name	Country	Process
	Tamales	Latin America	Lime-cooked corn is ground into dough that is mixed with lard, salt, chicken broth, baking powder, and spices. A small portion of the dough is spread onto soaked corn husks or cooked banana leaves and filled with spicy beans, meats, fish, or cheese. The tamales are wrapped and steam cooked for 60–90 min. More than 20 types of tamales are produced in Mexico.
Breads			
Unfermented			
	Tortillas	Mexico, Central America	Corn is lime (1% grain wt.) cooked for 15–60 min with three parts water at or near boiling temperature. The cooked kernels or nixtamal are steeped for 12–16 h and ground with stone mills into fine dough or masa. Small pieces of masa are hand or machine shaped into small pancake-like circles and baked on a hot griddle into tortillas. Tortillas and masa are the base for many traditional and regional dishes, i.e., tacos, enchiladas, sopes, joroch, totopos, nachos, tostadas, papusas, etc.
	Arepas	Venezuela	Moistened corn is dehulled and partially degerminated with the use of a wooden mortar and pestle (pilon). The resulting grits are cooked in boiling water and stone ground so that a dough is prepared. The final dough is hand-mixed with water to give proper consistency and seasoned with salt. Arepas are manually formed into flat disks (7.5 cm diameter × 1 cm thickness). Arepas are baked for approximately 2 min on each side on a clay or metal griddle called budare. Alternatively, uncooked Arepas are immersed in boiling water and then cooked on the budare or baked in an oven. Arepas are cut in half and stuffed with meats, cheese, butter, jellies, or other fillings. Arepas are also used for preparation of other foods (i.e., hallaquitas, hallacas, empanadas, etc.).
	Piki	U.S. (Hopis)	Prepared from a thin batter of blue corn meal, ash, and water. The batter is cooked on a hot flat stone to form a parchment like product. Piki can be crumbled, salted, roasted, and eaten like chips.
	Bivilviki	U.S. (Hopis)	The food is based on the same ingredients as Piki. Dough balls are rolled by hand and cooked in boiling water. The cooking water is also consumed with Bivilviki.
	Someviki	U.S. (Hopis)	Sugar-sweetened blue corn meal is mixed with ash and made into a dough that is wrapped in corn husks and boiled.
	Cornmeal	U.S. (Hopis)	Blue corn meal is added to eggs and baked in a skillet with shortening or drippings.
	Roti, Chapati	India	National bread of India produced from sorghum, millet, wheat, or corn. Corn flour (95–100% extraction) is mixed with hot water, kneaded into a dough, and hand-formed into a thin circular piece about 20–25 cm in diameter. The dough piece is cooked on a hot griddle at 210 °C for 25 s or until it puffs.
	Corn bread	Worldwide	Cornmeal alone or blended with wheat flour is processed into dough with water and/or milk and baked. Chemical leavening agents and flavorings (i.e., sugar, salt) are used in some formulations to obtain better texture, volume, and flavor. Many types of cornbread exist around the world.

Fermented			
	Injera	Ethiopia	Corn flour is mixed with water; a starter from a previous batch is used. The mixture is a thin, watery batter that is allowed to ferment for 17–72 h (first fermentation). A portion of the batter is mixed with three parts water and boiled. This is then returned to the main part of batter and the mixture is allowed to ferment for another 0.5–2 h (second fermentation). The leavened, acidic batter in a thin layer is then steam-baked on a covered clay griddle for 2–3 min over a very hot fire. The Injera is slightly shiny, soft, and flexible. It is characterized by the presence on the surface of uniformly distributed air bubbles or fish eyes.
Dough			
Unfermented			
	Masa	Mexico, Central America	Corn is cooked in lime or ashes to produce nixtamal. Nixtamal is stone-ground with a metate to produce a moist dough called masa. Masa is the backbone for production of numerous foods (i.e., tortillas, cornchips, tortilla chips, tamales, pozol, sopes, joroch, tostados, etc.).
Fermented			
	Kenkey	Ghana	Corn is soaked in water for 12–48 h, drained and ground. The dough is moistened with water and fermented for 2–3 days. After fermentation a portion of the fermented corn is half-cooked and blended with the remaining uncooked part. The product is salted, molded into balls, wrapped, and boiled until fully done. Kenkey is a sour dough that is used to produce a variety of similar products (i.e., Akasa, Koko, Banku, Akple, Abcle, and Kpekple).
	Pozol	Mexico	Lime-cooked corn is rinsed with water to remove pericarp. The nixtamal is ground into a coarse masa that is hand-shaped into balls. The balls are then wrapped into banana leaves and fermented for 1–14 days. Chorote is produced by adding ground cocoa beans to the masa before fermentation.
Alcoholic beverages			
	Urwaga. Mwenge	Kenya, Uganda	Corn flour is roasted over a fire. The roasted flour is blended with banana juice and then fermented for 12–24 h. The finished Urwaga is placed in smaller pots or jugs and drunk with a straw. It is a effervescent, brownish, slightly sour alcoholic beverage.
	Chicha	South America	Salivated or germinated corn flour and water (1:3) is heated to 75°C and thoroughly mixed for 1 h and then allowed to settle and cool. Three layers are formed: (1) the upper is liquid called Upi, (2) the middle with jelly-like consistency, and (3) the bottom part called Hanchi, which contains coarse particles. The Upi is place in another pot. The middle portion is concentrated to a sugar-like product. The Hanchi is pressed and filtered and the additional liquid is added to the Upi. The Upi may be simmered for several hours until it becomes caramelized. This product, called Misqui Kheta, is allowed to cool combine with more Upi, and fermented for 48–144 h to produce a clear, yellowish effervescent beverage called Chicha. The Chicha has to be consumed immediately; otherwise, acetic fermentation turns the product into vinegar.

continued

TABLE 11.1 (CONTINUED)
Traditional Foods Made with Corn

Food	Common Name	Country	Process
	Opaque beer, Chibuko	Southern Africa	Ground sorghum malt is mixed with water, allowed to sour, boiled with corn grits (adjuncts), cooled to 60°C, and saccharified with more sorghum malt. The mixture is filtered to remove coarse particles and fermented. The resulting beer is opaque, pink, and sour in taste (1–8% alcohol).
	Tesguino	Mexico	Corn is soaked in water for several days, drained and placed in a basket until it germinates. The germinated ground kernels extracted from corn stalks are boiled in water until the mixture turns yellow (8 h). The liquid is transferred to another pot where catalysts (ground leaves, beans, legumes) are added and fermented. Tesguino is a slurry-like alcoholic beverage.
	Pito	Nigeria	Corn is soaked in water for 2 days, drained, and held in a moist chamber for 5 days to germinate. The sprouted kernels can be used immediately or sun dried. The malted kernels are mashed, mixed with water, boiled 6–10 h, cooled, and sieved. The filtrate sours due to microbial fermentation. It is then concentrated by evaporation and cooled. Starter from a previous brew is added and allowed to ferment overnight. Pito is a light brown, alcoholic, slightly bitter, sweet-sour beverage.
	Talla	Ethiopia	A slurry of toasted, ground, and cooked corn flour is mixed with flavorings, pieces of freshly baked flat bread and wheat or barley malt. After a day, the mixture is diluted with water, fermented for 5–7 days and filtered. Talla has a smoky flavor and a tan to dark brown color.
	Busa	Kenya	Corn flour is mixed with water to form a stiff dough that is incubated for 3–4 days. The dough is disintegrated and toasted, mixed with water, and fermented for 3–4 more days. Then the mixture is filtered to produce Busa. The beverage is alcoholic, acidic with a light brown color.
	Opaque beer	Zambia	Corn is germinated for 3 days. The sprouts are alternately placed in the sun and stored. The dry malt is then pulverized. Corn meals are cooked to a thick porridge, cooled, and combined with the malt. The mixture is fermented for 1 day, cooled, mixed with more malt and fermented for 3 more days. The brew is filtered and consumed.
	Munkoyo	Zambia	Corn is cooked into a porridge and cooled. The Munkoyo extract (soaked root plant) is added. After 1 day, the product becomes sweet. The extract can be drunk without fermenting or allowed to sour for 2 days. The alcoholic beverage is similar to opaque beer.

Note: Data from Akingbala et al.,[20] Akinrele and Bassir,[21] Cuevas et al.,[22] Ekundayo,[23] Escobar,[24] Galiba et al.,[25] Gatumbi and Nuriru,[26] Harkishor,[27] Kuhnlein et al.,[28] Kunhnlein,[29] Lovelace,[30] Nyako,[31] Rooney et al.,[32] Rooney and Serna-Saldivar,[12] Serna-Saldivar et al.,[18] Smith et al.,[33] Steinkraus,[9] Taboada et al.,[34] Ulloa et al.,[35] Vivas et al.,[36] Vogel and Gobezie,[37] and Vogel et al.[38]

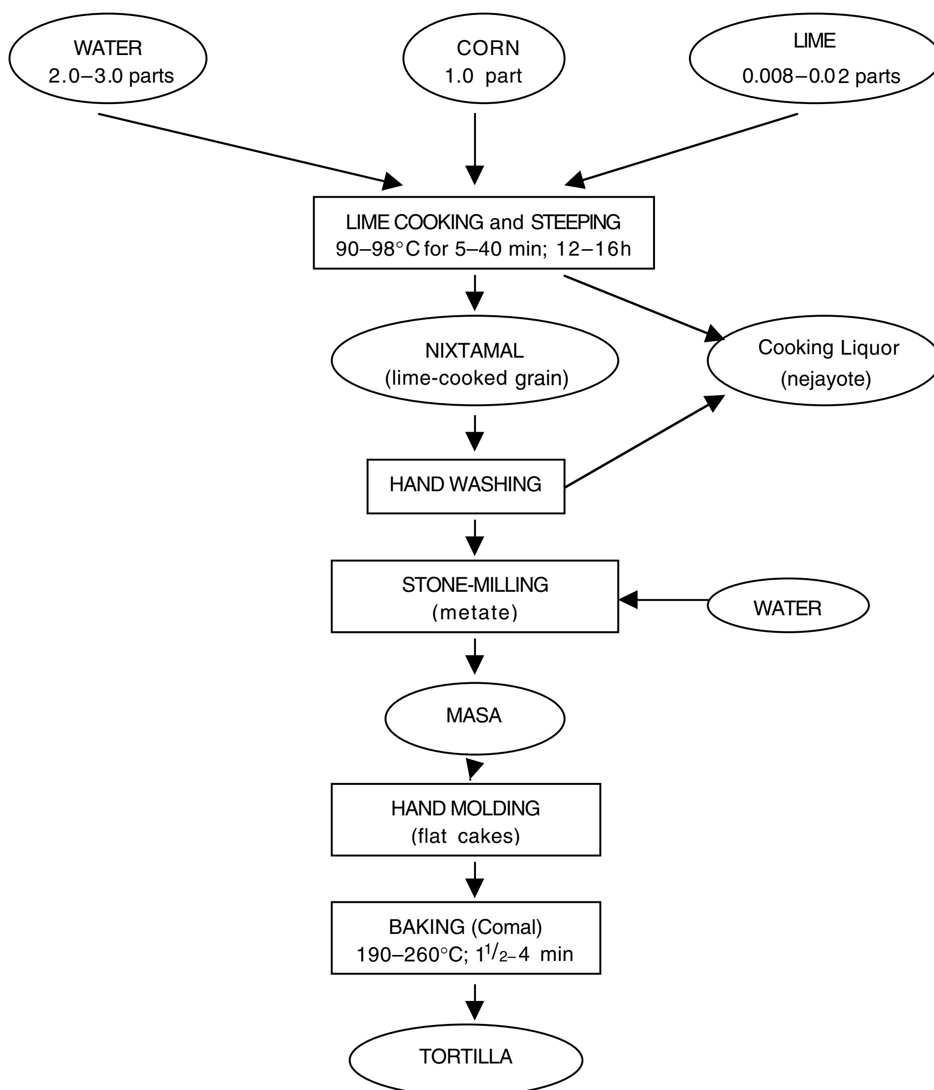


FIGURE 11.1 Flowchart of the traditional tortilla-making process. From Serna-Saldivar, S. O., Gomez, H. H., and Rooney, L. W., in *Advances in Cereal Science and Technology*, vol. 10, Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, MN, 1990. With permission.

0.2% crude fiber and 1% ash.[22] Arepas made from yellow corn have strong aroma and flavor, because the carotenoid pigments degrade during baking and frying. In some areas, yellow corn arepas are preferred, but generally white is most common.

In Mexico, Central America, and the Hopi area of the U.S., corn is heated on either hot stones or griddles until the kernel partially expands and develops brown color and roasted flavor. The parched corn is stone ground and used for gruels, porridges, and other products. Pinole is a breakfast gruel flavored with spices (cinnamon, anise) and brown sugar. Kernels are toasted on a griddle and ground to produce a shelf-stable pinole meal. The meal is blended with water and/or milk and boiled for a few minutes before consumption. Dry, shelf-stable pinole mixes are commercially available in Mexico.[18,36]

The Hopi Indians of the southwestern U.S. still consume traditional foods prepared with 12 to 14 different types of corn and in 70 different ways.[28,29] Many dishes are based on blue corn which

contains anthocyanidin pigments in the aleurone layer. Blue corn tortillas, chips, pozole, and other special products are sold in many restaurants, organic food stores, and some supermarkets. Blue corn products have a special flavor in addition to the color (Table 11.1).

The early settlers of North America cooked corn in lye or wood ashes to produce hominy. American Indians who taught the Colonists how to use wood ashes first used the process. The alkali effectively removes the pericarp, enhances the palatability and nutritional value of the corn kernels, and transforms the hard raw kernels into a soft, chewable product that can be stored relatively safely.[19] In the U.S., canned hominy is produced from white and yellow corn by using lye, which produces a different flavor than that of hominy prepared with lime. Corn for use in hominy is carefully dried and handled to avoid stress cracks. In Mexico, hominy is commonly used for the preparation of pozole (Table 11.1). Menudo is similar to pozole except the meat is from the stomach of ruminants.[18]

In Africa and Asia, corn is traditionally used for preparation of flat breads, beverages, and fermented or unfermented thick and thin porridges.[9] Many of the traditional foods are produced from fermented or germinated corn, which increases the vitamin content, mineral bioavailability, and protein quality. For example, the sorghum beer of Southern Africa often contains more corn grits than sorghum malt in the formula. Pap, Sadza, and Bogabe are popular everyday foods used in southern Africa. White maize is preferred to produce super-white meal or flour

IV. INDUSTRIAL PROCESSING OF CORN FOR FOODS

Most of the corn domestically used for foods is first processed by wet and dry milling industries. Wet milling produces relatively pure starch, protein, fiber, and germ. Dry milling produces "pure" endosperm fractions of varying particle size (grits, meal, and flour), germ for oil, and sometimes dietary fiber ingredients. Starch and refined dry-milled products are mainly used for the production of snacks, breakfast cereals, syrups, and alcohol. Alkaline cooking to produce dry masa flours, tortillas, and snack foods is increasing rapidly in the U.S. and other areas.

A. Whole Corn

1. Alkaline Cooked Products

In the U.S., the use of corn for tortilla chips and ethnic Mexican prepared foods has increased rapidly.[42,43] A similar situation is expected to occur in many other developed nations because of the demand for corn and tortilla chips. Nachos and many other foods based on corn are not only sold in bags but also compete successfully in restaurants, delis, amusement parks, and convenience stores. Corn snacks are relatively inexpensive and easier to process with greater flexibility and profitability than potato chips.[42] In 1998, total pound volume and sales of corn and tortilla chips captured 27.0 and 23.7% of the snack market share with $1.59 billion pounds and $4.35 billion in sales.[3] In contrast, potato chips sales totaled $4.59 billion and 25.2% of the market. Total pound volume and dollar sales of corn and tortilla chips increased 2.38 and 2.73 times, respectively, during the 1989–1998 decade. Regular and cheese-flavored tortilla chips continue to dominate the market. Corn and tortilla chips are becoming popular in other parts of the world as well. Processing plants have been started in England, Spain, France, Australia, Brazil, India, China, Korea, and other countries. Rooney and Serna-Saldivar[12] and Serna-Saldivar et al.[18] reviewed the chemistry and technology of tortillas, corn chips, and tortilla chips.

2. Industrial Tortilla Production

Today, even though most commercial plants use relatively sophisticated technology, the basic principles of the traditional tortilla-making process described before are used (Table 11.1; Figure 11.1). Commercial tortillas in the U.S. contain ingredients to improve product shelf-life and keeping

properties. The combination of acidulants (fumaric acid) and preservatives (i.e., sorbates and/or propionates) not only prolongs tortilla shelf-life but also affects typical flavor and aroma. CMC and other gums are used to bind water, retard staling, and enhance tortilla texture.[44] A general flow sheet summarizing typical processes for masa is presented in Figure 11.2. Lime cooking imparts the characteristic tortilla flavor, facilitates pericarp removal, controls microbial activity, enhances water uptake, increases gelatinization of starch granules, and improves nutritional value. Steeping distributes moisture and lime throughout the kernel. Stone grinding plays a key role, because it disrupts swollen starch granules and distributes the hydrated starch and protein around the ungelatinized portions of the corn endosperm, forming masa.[45]

Cooking and degree of grinding dictates the type of masa produced. Fine grinding produces masa suitable for table tortillas, while coarse masas are preferred for frying. Masa for table tortillas is usually more hydrated than masa for tortilla chips (55 vs. 51%).

Tortilla quality mainly depends upon the characteristics of the raw corn. The optimum grain should be sound, free of cracks and broken kernels with uniform size, and have intermediate to hard endosperm. Tortilla color is the result of corn kernel and cob color, the amount of lime used during cooking, the extent of washing, and the final pH. Kernels from red cobs can yield off-colored tortillas, because the tissue that remains after threshing is high in pigments.[46–48]

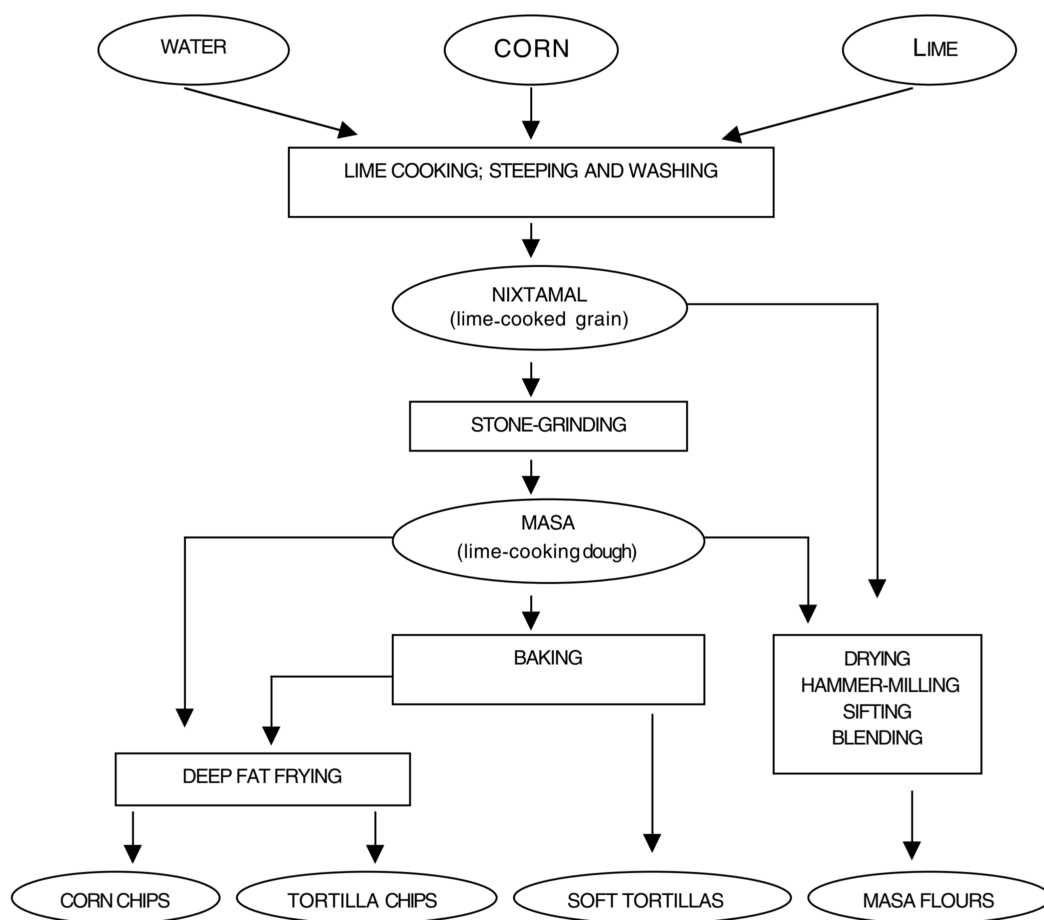


FIGURE 11.2 Flowchart of the main end uses of corn masa. From Serna-Saldivar, S. O., Gomez, H. H., and Rooney, L. W., in *Advances in Cereal Science and Technology*, vol. 10, Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, MN, 1990. With permission.

Important process variables are cooking time, temperature, kind and concentration of lime, and type of cooking equipment (size, agitation, and heating system). Corn is industrially cooked with three basic types of equipment. Open vats are generally used by small processors in the U.S. and are the most common type of equipment in Mexico. The method is energy inefficient and labor intensive, because the cooking vat is open and the mixture is manually agitated. Heating is accomplished with gas burners, although steam injector sparge tubes can substitute for the burners, making cooking more energy efficient. These vessels generally hold 180 to 900 kg of grain.[18,49]

The two more advanced systems are the Hamilton kettle and the vertical cooker. The Hamilton steam jacketed kettle is heated indirectly by steam and agitated mechanically. Corn is generally cooked at or near boiling temperature and immediately transferred into tanks for steeping. The vertical cookers employ direct steam injection to heat and agitate the corn and lime solution. The tank serves for both cooking and steeping. Additional agitation can be accomplished with compressed air. The later system is designed for cooking at temperatures well below boiling (i.e., 85°C). Thus, cooking time is generally longer than in steam kettles. The capacity of steam kettle and vertical cookers varies from 136 to 270 and 1360 to 2730 kg, respectively. These two systems are temperature controlled and can provide consistent results and high efficiency.

Corn is generally cooked with 2.5 to 3 parts water and 1% lime (may vary from 0.8 to 5% lime) based on grain weight (Figure 11.3). Cooking time varies greatly from a few minutes to 1.5

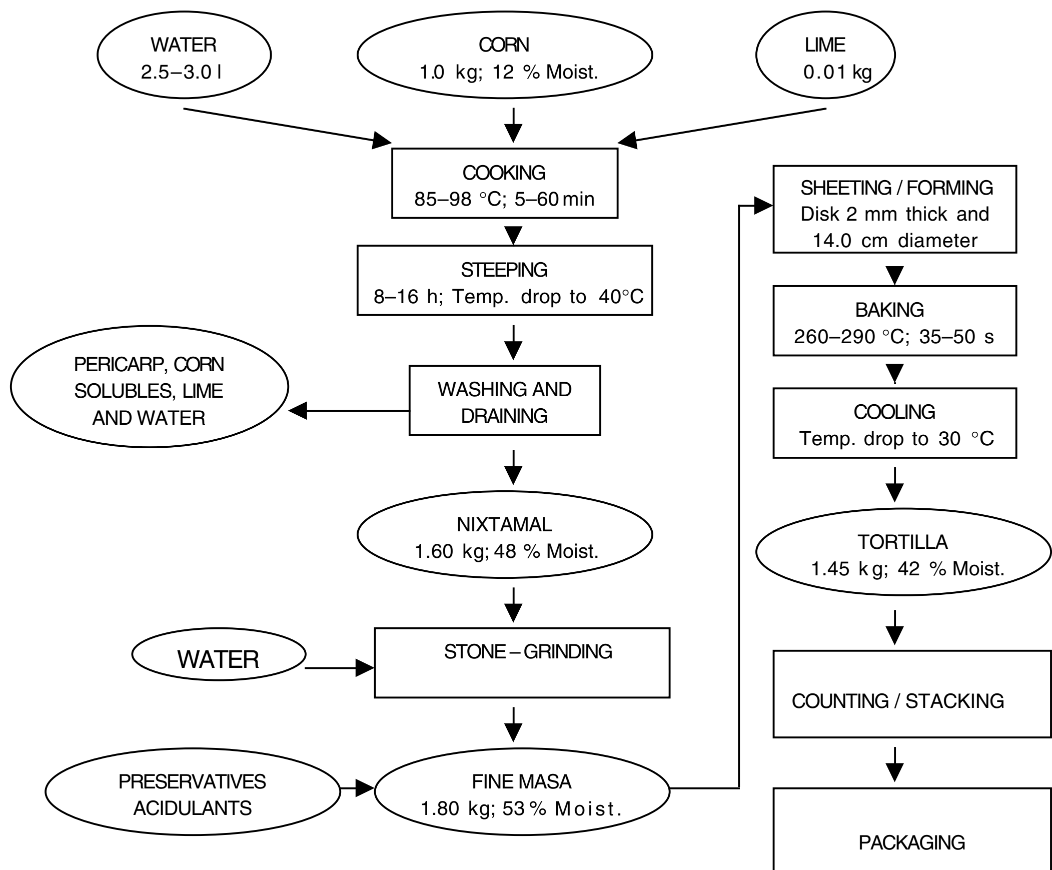


FIGURE 11.3 Flowchart of the commercial production of table tortillas. From Serna-Saldivar, S. O., Gomez, H. H., and Rooney, L. W., in *Advances in Cereal Science and Technology*, vol. 10, Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, MN, 1990. With permission.

h with 15 to 45 min as the most often-cited time. In general, temperatures above 68°C are thought to be required for cooking to occur. Cooking depends on the characteristics of the corn and the interaction of temperature, time, lime concentration, size of cooking vessel, and frequency of agitation. Optimum cooking and steeping are determined subjectively by evaluating the extent of pericarp removal, kernel softening, and overall appearance of the nixtamal. For table tortillas, longer cooking times are required and nixtamal is steeped without quenching. Nixtamal for corn and tortilla chips is cooked to a lesser extent either by decreasing cooking time or by quenching the steeping liquor to less than 68°C by addition of cold water.[18,49]

After steeping, the nixtamal and steeping liquor are pumped or dropped by gravity to mechanical washers. Most commercial washers are rotating barrels or drums that rinse the nixtamal with pressurized water. Pericarp and excess lime are washed away from the nixtamal. Pumping of the nixtamal increases dry matter losses during washing especially when poor-quality, stress-cracked, or chipped corn is used.[12,18,50,51]

The clean nixtamal is ground using a system of two matched carved stones, one stationary and the other rotating at 500 to 700 rpm. Volcanic and synthetic (aluminum oxide) stones are widely used by the industry. Synthetic stones have the advantage of lasting longer and require less recarving, but stones cannot be recarved in the tortilla plant. The typical stone is 10.2 cm thick, 40.6 cm in diameter, and is carved radially. The grooves become progressively shallower as they approach the perimeter of the stone. The number, design, and depth of the grooves varies with the intended product. For example, stones carved for table tortillas contain more shallow grooves so a finer masa can be produced. Stones for corn and tortilla chips contain fewer and deeper grooves. The grinding operation consists of forcing the nixtamal through a center opening that conducts into the gap between the stones. The material is cut, kneaded, and mashed while moving outward. Upon further kneading, the masa becomes plastic and cohesive. Water added during grinding cools the stones, prevents excessive wear, reduces masa temperature, and increases the moisture level in the masa. Masa particle size is the result of several interacting factors: (1) degree of nixtamal cooking; (2) carving of lava stones; (3) pressure between stones; (4) amount of water added during milling; and (5) type of corn. The yield of masa depends on the stones used, grinder capacity, and type of masa being produced. Grinders capable of milling 40 kg masa/HP/h are commonly used by the industry.[18,49]

In the U.S., masa is formed into tortillas using a head that consists of two rotating smooth Teflon-coated rolls that automatically presses the masa into a thin sheet. The gap between the rolls determines product thickness and weight. The resulting sheet of masa is cut by an attachment located underneath the front roll. A set of cutting wires also helps in discharging the pieces of masa into the conveying belt that feeds the oven. Different interchangeable cutter configurations are used for production of various products. Sheeting heads that cut single to eight rows of tortillas exist in the industry.[18,49]

In Mexico, most tortillas are formed with machines (celorio) that consist of a mixer, extruder, and former. The extrusion system forces the masa through a slot at the bottom of the unit. A gate cutter controls the discharge and regulates the shape and size of the masa product. This machine is exclusively used for table tortillas and requires a finely ground masa. The masa is usually hydrated to a larger extent (60% moisture). Tortillas extruded and formed with the these machines generally puff during baking and keep their textural properties longer.[18]

Newly formed masa pieces are baked into tortillas on a triple-pass, gas-fired oven at temperatures ranging from 280 to 302°C for 20 to 40 s. The baked tortillas are cooled for 3 to 5 min through a series of open tiers that discharge into the packaging area. Tortillas are counted, stacked, and bagged in plastic materials.

3. Fried Products — Snacks

The majority of the lime-cooked corn products consumed in the U.S. is in the form of fried snacks. Corn and tortilla chips produced from coarse masa are the most popular products (Figure 11.4). In

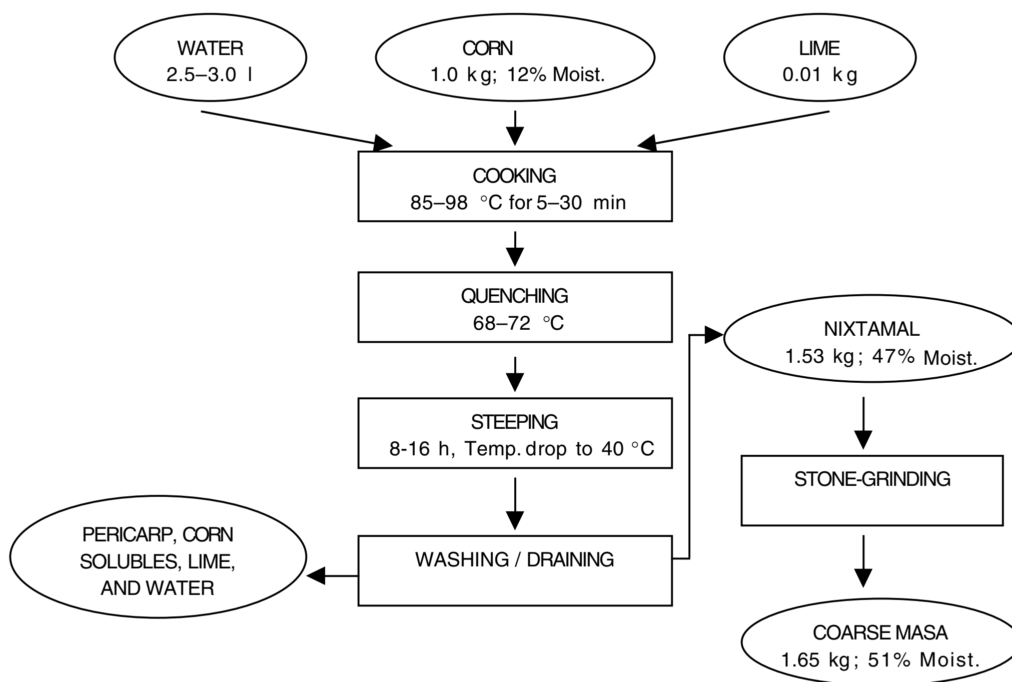


FIGURE 11.4 Flowchart of the process for producing coarse masa for corn and tortilla chips. From Serna-Saldivar, S. O., Gomez, H. H., and Rooney, L. W., in *Advances in Cereal Science and Technology*, vol. 10, Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, MN, 1990. With permission.

contrast to table tortillas, corn is cooked less and/or quenched immediately after cooking and ground into a coarse, less hydrated masa. For corn chips, the masa is formed and directly fried. For tortilla chips, masa pieces are baked before frying.[12,18,52]

The fryers are designed to maintain uniform temperature and produce products with acceptable color and low moisture content (<2.5%). Most commercial fryers are continuous with direct or indirect heating elements. Frying temperature and product residence time depend on the type of product. Masa or tortilla pieces from yellow maize require lower frying temperature than pieces from white or blends.[12,18]

Corn chips contain more oil (32 to 38%) than tortilla chips (21 to 24%). This large difference is due to the moisture content of the unfried product. Masa strips for corn chips contain at least 48 to 50% moisture, whereas the baked tortilla chips contain 38 to 42% moisture. For production of taco shells, tostadas, baskets, and related products, tortillas from coarse masa are either bent or formed into the desired configuration using special forming devices and then fried (Figure 11.5).[18]

Salt and flavoring agents are applied onto tortilla and corn chips immediately after frying with rotating cylinders or drums equipped with powder dispensers or spraying systems. Then, fried products are immediately packaged in moisture-proof bags, because chips absorb moisture readily with consequent loss of crispness.[18]

Many processors are developing "reduced fat" fried products for special markets. This is being achieved by modifying manufacturing procedures in which the product is baked or toasted. Ellis and Friedemann[53] hold a patent for production of low-oil, masa snack foods (i.e., corn chips and tortilla chips) from waxy corn or blends of waxy and regular dent corn. Waxy corn kernels require less cooking and steeping than dent corns. For production of reduced-oil snacks, moisture from the shaped masa or baked tortilla chip is removed by further baking or toasting (180°C for about 3 min) or baking/flash frying (207°C for only 7 to 10 s) to yield a product with less than 2%

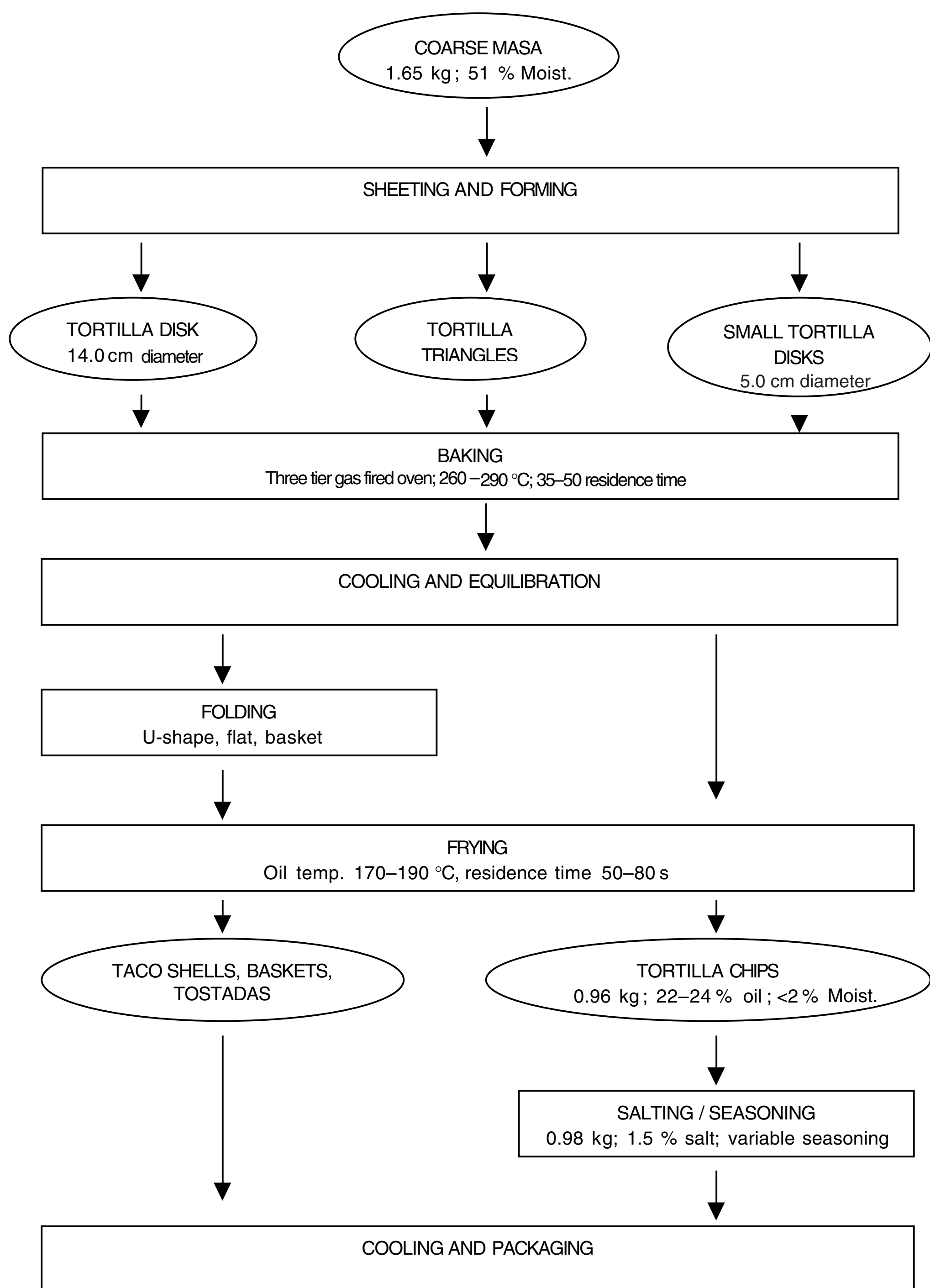


FIGURE 11.5 Flowchart of the process for producing tortilla chips and related products. From Serna-Saldivar, S. O., Gomez, H. H., and Rooney, L. W., in *Advances in Cereal Science and Technology*, vol. 10, Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, MN, 1990. With permission.

moisture. Toasted tortilla chips are sprayed with cooking oil to increase oil content to 7 to 8% and enhance their organoleptic properties. The texture and flavor of reduced oil tortilla chips differ

from conventional counterparts. According to Lee,[54] waxy corn requires a significantly lower cooking time and special grinding conditions to prevent the formation of sticky masa. Baking produces sticky raw tortilla chips that, upon frying, yield a spongy-textured chip. Recently, Quintero-Fuentes et al.[55] utilized an air impingement oven to produce baked corn and tortilla chips. Masa triangles were baked in an air impingement oven to produce baked corn chips, or first baked in a three-tier oven and then in an air impingement oven to produce baked tortilla chips. The utilization of waxy rice, maize or sorghum flour helped to develop a porous structure with numerous small internal air cells. The air impingement baking procedure proved to be useful to produce nixtamalized baked chips.

Blue corn has been traditionally used for tortilla and chips in New Mexico and Arizona. Today, some commercial factories are manufacturing blue corn products for speciality markets. The products have a blue coloration and a unique flavor. The soft textured kernels of blue corn must be cooked for a short time if good quality products are desired. A similar situation occurs when *opaque* 2 kernels are processed into tortillas. New QPM corns process similarly to commercial yellow and white corns for tortilla production.[18,56,57]

4. Nixtamalized Dry Masa Flours

The use of dry masa flour for in-home tortilla preparation in Mexico and for commercial use in the U.S. is expanding rapidly. Several companies produce nixtamalized flours that need only to be rehydrated to produce masa. Dry masa flour has a long shelf-life (up to 1 year in dry storage); its use eliminates the tedious, labor-intensive cooking, washing, and grinding. In addition, processors do not have to worry about effluent disposal, the selection of suitable corn, and managing its cooking.[18]

Industrial production of dry masa flours is accomplished by lime cooking, washing, and grinding the corn to produce masa, followed by drying, grinding, sieving, classifying, and blending to meet certain requirements[52,58] (Figure 11.6). The dried masa is formulated into flours with carefully controlled particle size distribution. Montemayor and Rubio[59] described continuous and batch cooking procedures for production of masa flours. In a continuous process, the lime (0.5 to 1% based on corn weight) is mixed with equal parts of water and corn in a large screw conveyor fitted with steam jets. The corn cooks while moving along the conveyor. The nixtamal is washed to remove pericarp and excess lime and ground with stone or hammer mills. The ground particles are flash-dried and sifted, and large particles are ground and classified into the different fractions. The different particle size fractions are blended to meet desired applications. In batch processes, the corn is mixed with lime, cooked, steeped, washed, ground with hammer or stone mills, dried, ground, sifted, and formulated into dry masa.

The strong demand for Mexican foods in the U.S. has increased the number of types of dry masa flours available. Masa flours for production of white and yellow table tortillas, restaurant-style tortilla chips, tortilla chips, corn chips, and tamales are available. Some companies offer more than 25 different masa flours formulated to meet certain color, pH, particle size distribution, water absorption, and viscosity requirements. In general, the particle size distribution is coarser for snacks, taco shells, and tostadas, because pores are needed to vent steam during frying. Various additives, such as gums, acidulants, and preservatives are used in many masa flours for table tortillas to enhance tortilla texture and shelf-life.[12,18,52,58]

Alternative methods to produce masa flours have been proposed. The aim of these methods is to produce nixtamalized dry flours continuously, faster and more efficiently in terms of labor, energy, and floor space. Extrusion cooking has been used as one of the alternative methods. Excessive starch gelatinization and extrudate puffing are avoided by controlling grit size and moisture content, screw configuration, speed, and heat input. Generally, corn grits are mixed with 0.2 to 0.3% lime and water to reach a moisture content of 34%. The tempered blend is continuously fed into the extruder and exits at a moisture content of approximately 18 to 20%. An additional

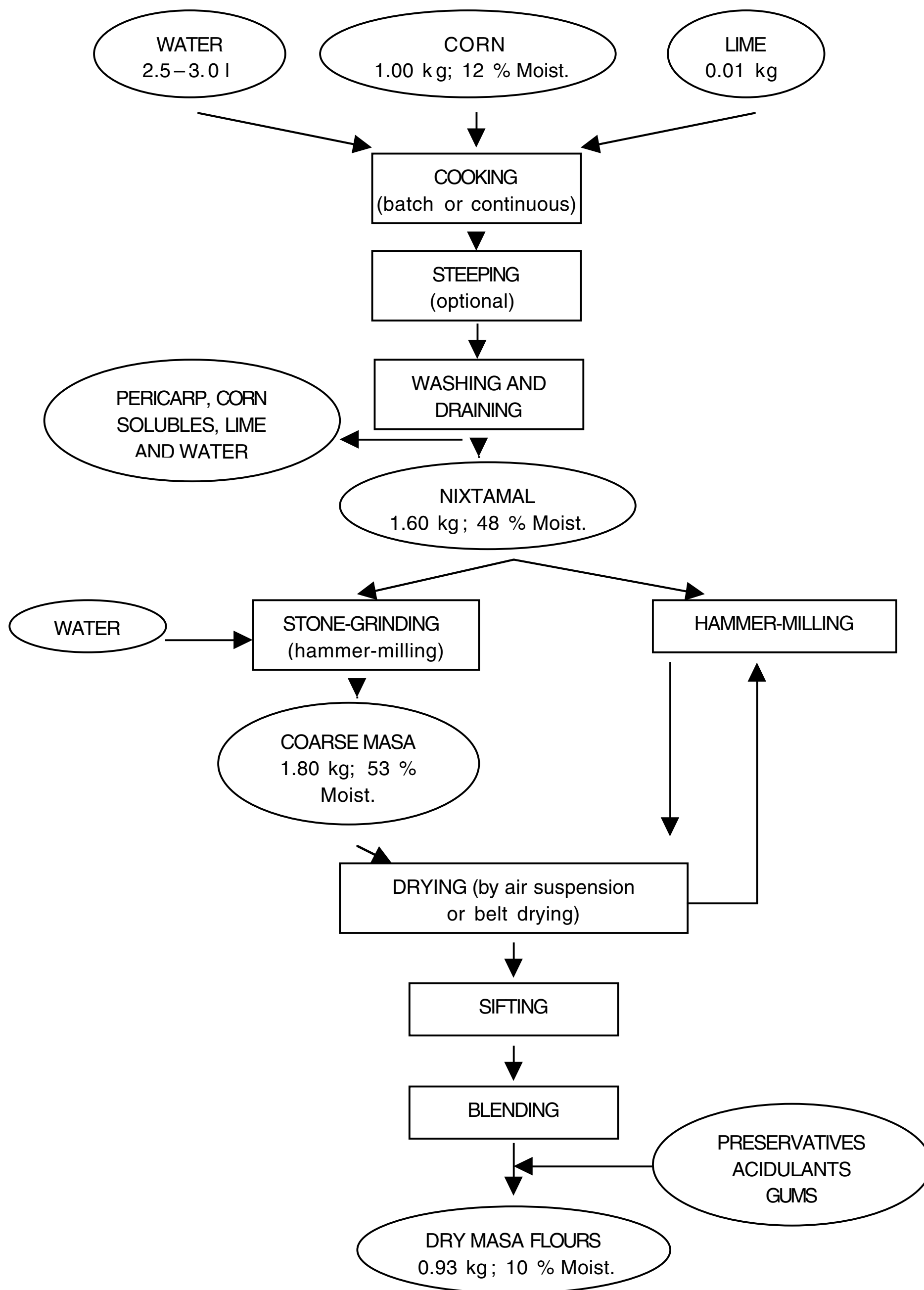


FIGURE 11.6 Flowchart of the industrial process for production of dry masa flour. From Serna-Saldivar, S. O., Gomez, H. H., and Rooney, L. W., in *Advances in Cereal Science and Technology*, vol. 10, Pomeranz, Y., Ed., American Association of Cereal Chemists, St. Paul, MN, 1990. With permission.

10% moisture is removed by a continuous drying operation at 65°C. The extrudate with 10% moisture is hammer-milled into flour, which is further classified by particle size and reblended.[60–61]

The use of a drum drier to produce masa flour was proposed by Molina et al.[62] Whole corn flour is mixed with water and lime and simultaneously cooked and dried in a double drum with a

gap of 0.007 mm and an internal pressure of 110 to 183 kg/m^2 at 2 to 4 rpm. Supposedly, the tortillas produced by the flour from drum drying have properties similar to those produced by the traditional method.

Micronizing (dry heat treatment with infrared lamps) of corn grits previously tempered with dilute lime solution has also been proposed for production of dry masa flour. The tempered grits are cooked, flaked, cooled, and ground into flour.[63] Hart[64] patented the production of masa flour using micronization. A similar process was developed by Villalba[65] in which corn kernels tempered in a lime solution were dry-cooked with a jet sweep impingement oven.

All of these methods produce dry masa flours with relatively poor quality that is undesirable. A few commercial dry masa flours are produced from corn dry-milled fractions that are treated with heat and alkali. Some of these produce acceptable tortilla chips and taco shells. They do not make good quality table tortillas; research continues to further refine these flours to produce acceptable table tortillas.

5. Hominy

The industrial preparation of hominy includes the use of caustic soda to soften and remove the pericarp. A hot water solution containing 0.86% lye is added to cover the corn, which is periodically stirred while cooking at boiling for 25 to 40 min. The hominy is washed with water to remove pericarp and traces of lye.[19] Hominy is often salted and canned and used as an ingredient in soups and salads and as a vegetable.[12] Spanish-style hominy uses lime to cook the corn.

Hominy is made from both yellow and white corns, although the preference is for white hominy. Varieties that produce large kernels with hard endosperm are preferred for lye cooking. Damaged kernels with cracks and fissures process poorly because they disintegrate during cooking, washing, and canning. Thus, the specifications for corn used to produce hominy are very rigid; field-dried corn is preferred. For pozole, large, floury corn kernels are preferred.

6. Cornnuts and Parched Products

The corn for Cornnuts™ was developed from the Cuzco Gigante race from Peru. The eight-rowed ears produce the largest known corn kernels.[66] Cuzco corn grows at high altitudes and produces white kernels with soft endosperm texture and bland flavor. The first Cornnut hybrid adapted to the U.S. was introduced in 1964 after 9 years of painstaking breeding work. Several new hybrids have been developed since then. Kernels are harvested when the moisture content of the grain is less than 30%. The kernels are cleaned, sized, and dried to reduce moisture to less than 15%.

Preparation of Cornnuts starts when kernels are heated in alkali and washed to remove the pericarp. Once peeled, the corn is transferred into soak tanks, where it is steeped for a few hours in warm water. The hydrated kernels are fried to develop the characteristic texture, flavor, and color. The final product contains about 14% oil. Cornnuts that do not meet color quality control requirements are removed; finally, the kernels are flavored and packaged.

V. INDUSTRIAL DRY MILLING PROCESS

The U.S. dry milling industry processes annually more than 4 million tonnes or about 2% of the total corn produced in the U.S. There are approximately 71 dry corn mills in 22 states.[2] A few larger millers produce a very high percentage of dry milled products. Dry millers produce a wide array of products for food, feed, and industrial uses. Baking, batter, dry mix, fast food, snack food, breakfast cereal, and brewing industries are major customers of corn dry millers.

Dry millers process corn in two ways: by stone grinding the kernels to produce hominy grits and whole meals rich in bran and germ and by degermination processes. The latter process produces highly refined grits, meals, and flours with extended shelf-life.

Fresh corn meals have rich flavor because of their high oil and germ content. Some meals are bolted to remove coarse particles of bran and germ. Anderson and Watson[67] report the average chemical composition of these products. Whole or bolted meals have a shorter shelf-life because the oil becomes rancid quickly. Generally, white corn is preferred for production of whole meals, especially in the southern U.S.

Watson,[15] Anderson and Watson,[67] Alexander,[68] and Brekke[69] have described the detailed tempering-degerming process for corn (Figure 11.7). U.S. No. 2 yellow dent corn is the type most frequently used by millers. About 25% (30 million bushels) of the total corn dry milled in 1987/88 was white.[2] Kernel hardness and freedom from stress cracks are the major quality criteria for corn used in dry milling.[48] High-temperature drying creates stress cracks that lead to breakage during handling.[70] Dry milling quality decreases linearly with increasing drying temperature.[71] Kirleis and Stroshine[71] found that hard hybrids had better milling characteristics than soft counterparts. Density was the grain physical factor more closely related to milling yields. For high yield of flaking grits, corns with high test weight and hardness are recommended.[72]

The objective of dry milling is to produce the maximum percentage of clean grits, containing minimum fat, fiber, and specks from the hilum and to recover the maximum percentage of clean germ with maximum oil content and largest particle size.[73,74] The corn is thoroughly cleaned by combinations of sieving, aspiration, washing in water, electrostatic separation, and other methods (Figure 11.7). All mills carefully examine incoming lots of corn for aflatoxins and grain molds. The clean corn is conditioned to 20 to 23% moisture and placed in a tempering bin for 1 to 3 h.

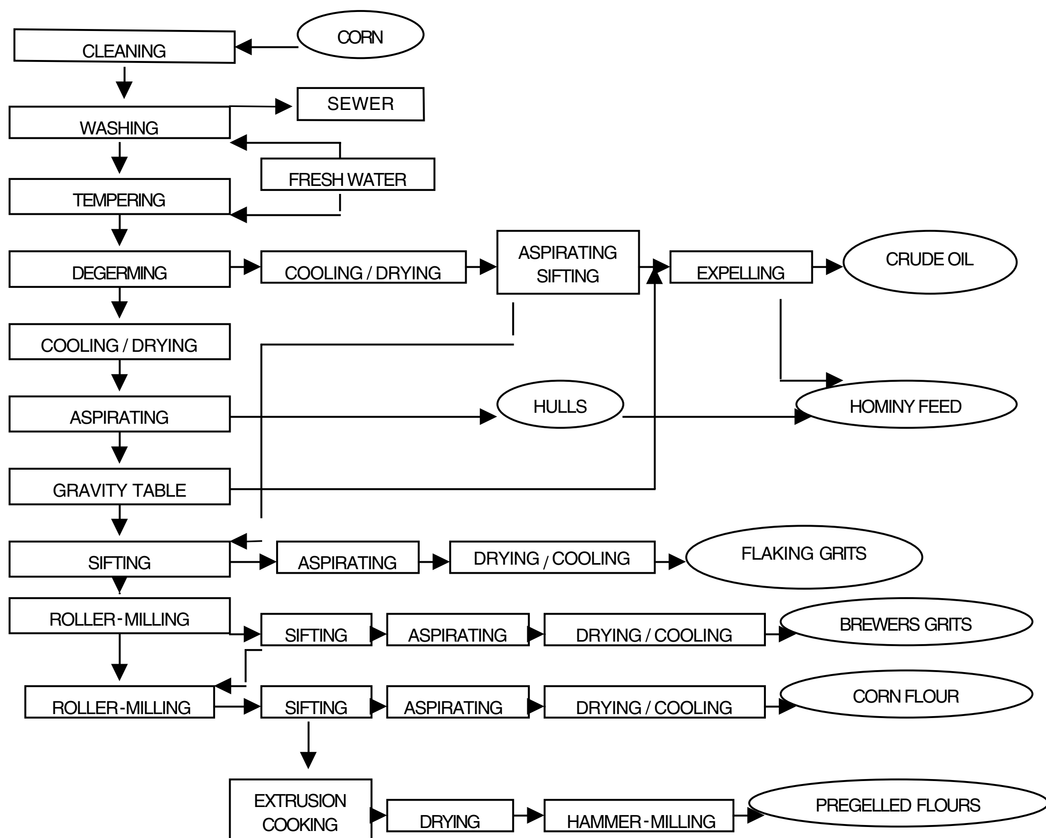


FIGURE 11.7 Flowchart of the industrial corn dry milling process. (Adapted from Watson, S. A., *Corn and Corn Improvement*, Sprague, G. F., Ed., Agronomy No. 18, American Society of Agronomy, Madison, WI, 1977. With permission.)

The objectives of conditioning are to toughen the germ and bran to facilitate subsequent separations. Tempering hydrates the endosperm so maximum grit yields with minimum flour is achieved.

Degermination is the key to efficient dry milling. The most common degerminator is the Beall, first introduced in 1906. It consists of a conical knobbed rotor and a stator shell that is knobbed on the lower convex surface and slotted on the upper surface. With the Beall degerminator most of the germ and pericarp are removed. The degerminator is set to produce large, clean pieces of endosperm known as hominy tails. This endosperm fraction is partially dried (15% moisture), cooled, and reprocessed to obtain flaking grits, medium and fine grits, meal, and flour. Reduction of the large endosperm pieces is done with roller mills followed by sifting and aspiration to remove bran particles from the grits. Then, gravity tables separate germ pieces from the endosperm particles. Endosperm fractions are finished in purifiers designed to remove fine pieces of pericarp and are packaged at a moisture content of 12%.[75] Typical yields of dry milled fractions are in Table 11.2. The mean chemical composition of these products has been reported elsewhere.[15,67–69]

The main byproducts of the dry milling industry are germ and bran. In most cases, germ is pressed and/or solvent extracted to yield crude oil and defatted germ meal. Dry milled corn germ has excellent nutritional value and has been proposed for human foods. Corn bran fractions are used in prepared foods to increase dietary fiber content.[76] Different particle sizes, from coarse to ultrafine, are manufactured for specific applications. The most desirable bran product should be relatively high in total dietary fiber and low in oil, protein, and starch. Burge and Duensing[76] reported typical analysis of corn bran and its fiber composition. Today, corn bran is used to produce high-fiber, low-calorie foods and to enhance moisture retention in various food systems.

One of the main reasons for the failure of the *opaque 2* maize was due to its poor dry milling properties. Van Twisk et al.[77] in South Africa, Brekke et al.,[78] and Wichser[73] determined that the soft kernels of *opaque 2* corn yielded fewer grits with much finer particle size distribution than normal corn. To mill *opaque 2* corn, several adjustments in the process and problems were encountered: (1) kernels had to be tempered to around 14% moisture[73] or more than 23% moisture[78] instead of the common 20 to 23%; (2) the bran of *opaque 2* was more difficult to separate because it was tougher and remained attached to the endosperm chunks and germ after degermination; and (3) the separation of endosperm pieces from germ in gravity tables was more difficult because the floury endosperm had almost the same density as the germ.[73,78]

Wu[79,80] and Kirleis[81] compared the dry milling properties of quality protein maize (QPM) with conventional corn. Yields of grits and prime products from QPM were similar to regular dent corn. The lysine and tryptophan composition of the QPM's dry milled fractions was significantly higher

TABLE 11.2
Typical Yields of Corn Dry-Milled Fractions

Product	Yield (%)
Flaking grits	12
Coarse grits	15
Regular grits	23
Coarse meal	3
Dusted meal	3
Flour	4
Oil	1
Hominy feed	35
Shrinkage	4

Source: Brekke, O. L.[69] With permission.

than corresponding fractions from regular corn. The density of QPM was positively correlated with total grits yield.

Serna-Saldivar and Rooney[82] and Serna-Saldivar et al.[57] recently reviewed the industrial utilization of QPM. The development of high-producing QPM varieties and hybrids has been achieved in Brazil, Ghana, China, South Africa, and other countries around the world. Brazil is commercially planting two varieties: a white corn named BR 451 released in 1988 and a yellow corn BR 473 released in 1994. These varieties are agronomically competitive with some of the more popular early maturing normal varieties cultivated. The CNPMS/EMBRAPA is working on the development of hybrids using inbred lines derived from BR 473.[83] QPM open-pollinated varieties and hybrids that perform better than normal corn have been developed in Ghana. The QPM variety named 'Obatanpa' has received wide acceptance. Currently, over 50% of seed sales are from this highly nutritious corn. Human and animal nutrition studies have shown the nutritional advantage of this QPM over normal corn.[84] The utilization of QPM in these countries can have a significant social impact, since malnutrition remains a major problem that plagues a portion of the rural and urban populations. The swine and poultry industries also can benefit from QPM.

VI. FOOD USES OF DRY MILLED FRACTIONS

Endosperm products from corn dry milling (Table 11.2), ranging from large grits to flour, are widely used by brewers, snack food, and breakfast cereal processors. Corn endosperm fractions are cooked to varying degrees with different types of equipment to obtain many different products and intermediate ingredients.

A. Flaking Grits: Cornflake Production

Flaking grits are large (US 3.5–6 mesh sieves) endosperm chunks obtained after corn degerming. These large pieces of endosperm are almost exclusively used to manufacture cornflakes, the most popular ready-to-eat breakfast cereal in the world.

The fundamental process for cornflake production (Figure 11.8) has remained relatively unchanged over the past century. Yellow corn with uniform kernel size and unfissured hard endosperm is preferred for flake production because of its stronger flavor and rich golden color after toasting. The flaking grits are pressure-cooked in rotary cookers at 0.044 to 0.067 tonnes/cm^2 for 1 to 2 h with syrup, sugar, nondiastatic malt, salt, and water. The grits reach 28 to 33% moisture and appear translucent when properly cooked. The starch is gelatinized during cooking, but granule swelling is restricted due to limited availability of water. After the cooking cycle is completed, the grits are conveyed by a moving belt to equipment that breaks apart grits that are stuck together. Then, the cooked grits are transported to a countercurrent dryer set at 66°C for 2.5 to 3 h. Residence time is set so the grits exit the dryer at 19 to 20% moisture. Then, the grits are equilibrated for 6 to 24 h in a bin. Tempering allows for better moisture equilibration and reduces the chances of corn flake breakage during handling. Grits are flaked through a pair of counterrotating rollers which apply a pressure of 234 tonnes/cm^2. The resulting soft flakes are toasted in a gas fired oven for 50 s at 302°C or for 2 to 3 min at 288°C. Toasting dehydrates the flakes, develops a crisp texture, brown color, and the characteristic flavor. Finished flake moisture content is approximately 2%. Upon cooling, the flakes are sprayed with nutrients and equilibrated. Sugar-coated cornflakes are made by the same process, but the sweetener is sprayed onto the flakes after toasting.[12,16,85]

Cornflakes can be made from corn meal or grits that are extruded to produce pellets. Pellets are dried, tempered, flaked, and toasted, as described in traditional cornflakes.[16,85]

Midden[86] proposed a technology to produce cornflakes using twin screw extrusion. Ingredients are batch mixed and then fed into the extruder, where the mixture is cooked, cooled, and processed into spaghetti-like strands. The extruder operates at 150°C in the cooking section and 25°C in the

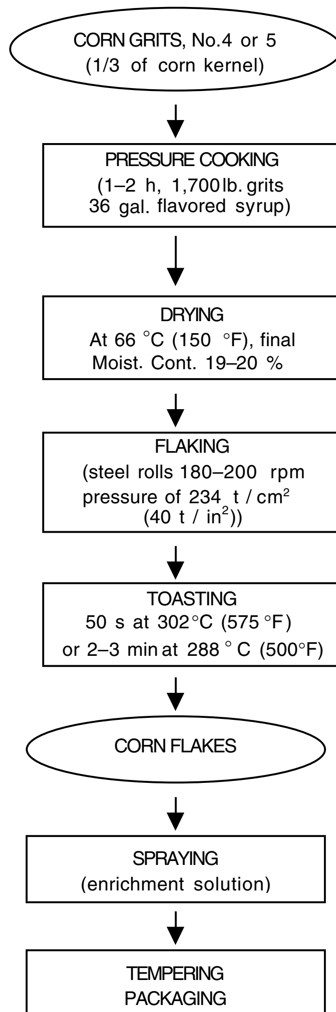


FIGURE 11.8 Flowchart of the industrial process for production of cornflakes.

cooling section. The end result is a dense extrudate that is cut into 5-mm-long pellets. Cooked pellets are flaked and toasted in the same way as in the traditional method. The advantages of this new process is that flakes are produced in 30 min instead of 8 h required in traditional cornflakes. These products have uniform flake size and 11% savings in costs, but the texture differs significantly from conventional cornflakes.

B. Corn Grits

Corn grits are particles of endosperm that pass through a 1.19 mm sieve (US No. 14) and ride on a 0.59 mm sieve (US No. 28). They are low in fiber and contain less than 1% oil. Grits of various granulations are widely utilized by the snack, breakfast cereal, and brewing industries. In the U.S., corn grits are consumed as a side dish for breakfast. The grits are cooked in boiling water for 10 to 25 min and then seasoned with butter or margarine. Instant or precooked corn grits, which require only 5 min cooking, are popular. In southern states, white corn grits are preferred over the yellow counterpart because they possess a bland, sweeter corn flavor.[87]

1. Brewing

Traditionally, the brewing industry has been the largest user of corn dry milled products. Grits are used as a source of inexpensive fermentable carbohydrates. Brewers grits have a size range from 10 to 12 mesh, which facilitates filtration of the wort from spent grains after mashing. Brewers grits are cooked to hydrate and gelatinize the starch and then treated with malt. Starch from both the malt and grits is broken down to fermentable sugars by the enzymatic action of amylases. After hydrolysis, the solubilized carbohydrates are separated from the spent grains by filtration to produce wort. The residue of the grits after hydrolysis play an important role in the ease of wort filtration. An excess of fine grit particles can increase run off time during lautering.[88] The most desirable grits contain less than 1% oil, ash, and fiber, have optimum particle size distribution, and high malt extracts. The wort is hopped and inoculated with yeast to produce green beer. The green beer is allowed to age for 7 to 50 days. Then it is filtered, carbonated, pasteurized, and bottled.

2. Distillation

Whole ground corn and corn grits are used to produce vodka, whiskey, and many other alcoholic beverages. A flowchart of a typical distillation process for production of whiskey is presented in Figure 11.9.[89] Straight bourbon whiskey is obtained from distillation of a fermented mash containing at least 51% corn in the U.S.[90] The distillate is aged for 2 to 10 years in wooden barrels, blended, and bottled. Bourbon whiskey originated in Bourbon County, Kentucky.

3. Breakfast Foods

Annual consumption of ready-to-eat breakfast cereals has steadily increased in the U.S. during the 1986–1996 decade.[85] Frosted Flakes is still the top cereal brand in the U.S. with yearly sales of approximately $330 million. In 1996, the average American spent more than $30 for the purchase of breakfast cereals and consumed on average 10 packages weighing about 10 lb. The cereal industry has grown to a volume of over $8.3 billion.[85] Breakfast cereal consumption continues to increase due to the increased number of working mothers, the convenience of these foods, advertising, and the increased interest in dietary fiber and enriched/fortified foods.

Corn alone or in combination with other cereals and ingredients is often used in ready-to-eat breakfast foods. Corn grits, meal, or flours are cooked to gelatinize the starch, denature the protein, and produce a dough that can be processed into flakes, shreds, granules, puffs, or collets. Desirable flavor, aroma, and texture are usually obtained by controlled toasting.

Currently, most new breakfast cereals are prepared by continuous extrusion and puffing, which has economic advantages.[91] Extruders are often used in the production of breakfast and snack foods. Extruders most often used include (1) forming extruders that size and shape a precooked dough; (2) extruders that mix and cook cereal-based ingredients at ordinary pressure and then form and size the dough; and (3) short-time, high-temperature continuous cooking and puffing extruders.

Many breakfast and snack foods are produced by short-time, high-temperature, high-pressure extrusion cooking and puffing (Figure 11.10). Corn flour or meal is moisturized, and combined with starches, flavorings, and coloring agents to prepare a wide array of products. Extrusion puffing offers the advantage of producing many products with different characteristics. Ingredient formulation, granulation, additives, and extruder working conditions (temperature, dwell time, length pH, moisture content, rpm, type of screw, and die characteristics) determine the type and characteristics of product manufactured. Degree of expansion depends on the pressure difference between the dough inside the extruder and the atmosphere outside. Shapes and sizes of products are varied by changing the die and the cutting blade spread. The expanded extrudate can be dried-toasted, coated, and flavored.

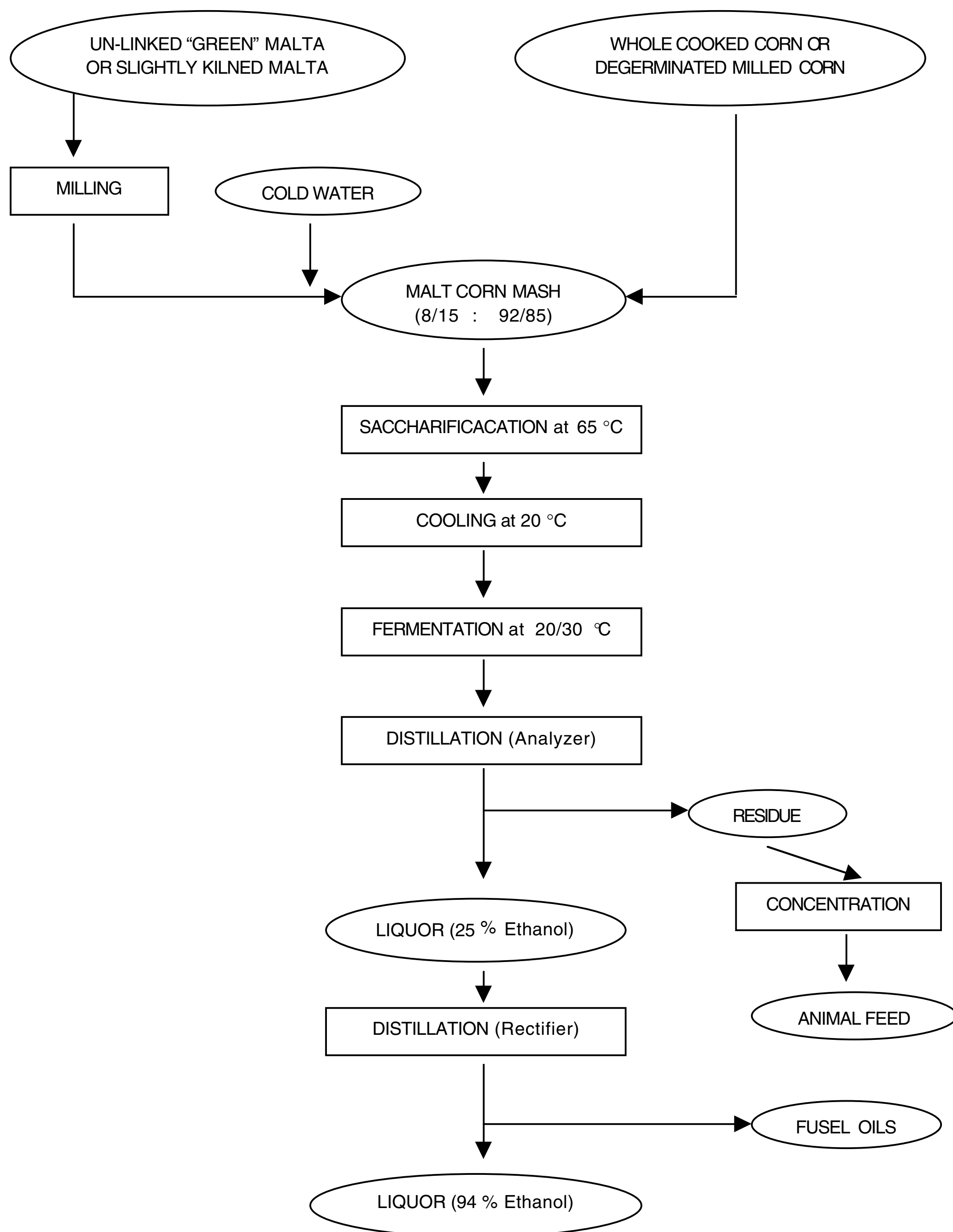


FIGURE 11.9 Production of distilled alcohol beverage from corn. (Adapted from Bathgate, G. N., *Cereal Science and Technology*, Palmer, G. H., Eds., Aberdeen University Press, Aberdeen, Scotland, 1989. With permission.)

Extruded gun puffed cereals are still one of the most popular types of ready-to-eat breakfast cereals (Figure 11.10). Corn flour, meal, or small grits are cooked in combination with other cereals, sweeteners, flavoring, and coloring agents into a dough that is formed through a die and cut into the final shape. The extrudate, called a collet, is dried to an optimum moisture content before gun

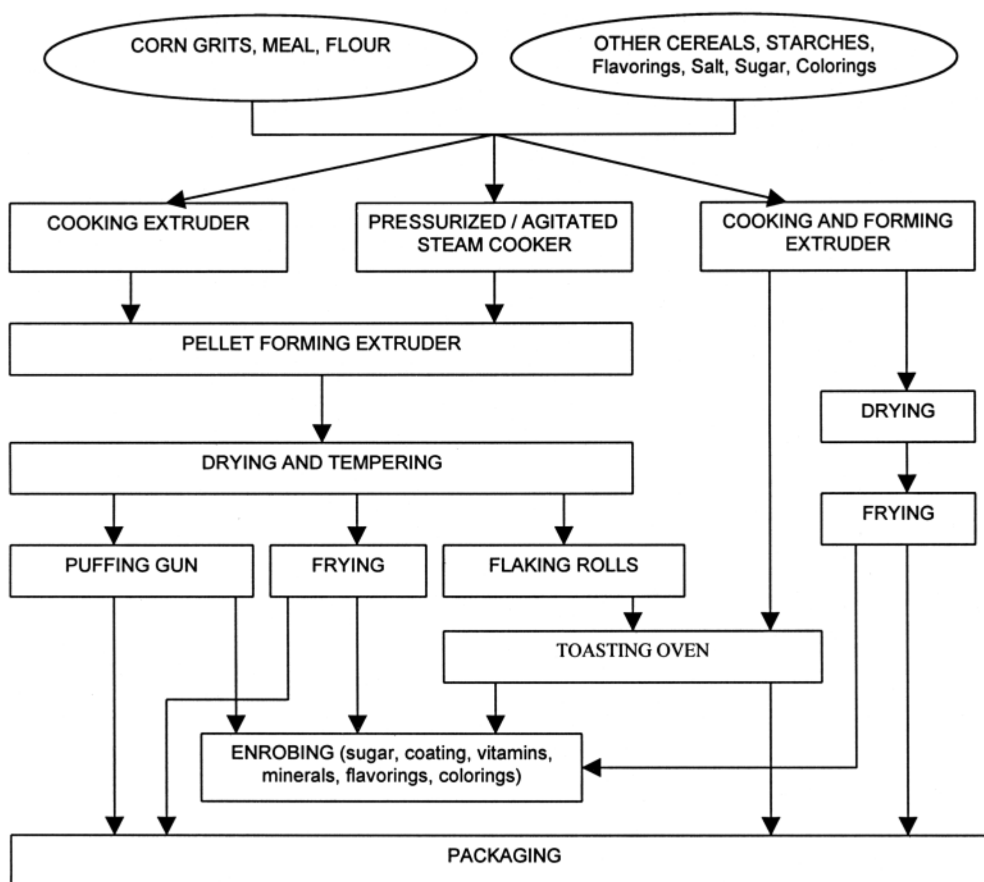


FIGURE 11.10 General scheme for the production of breakfast cereals and snacks via extrusion cooking.

puffing. The puffing gun is a pressure vessel approximately 15.2 cm in diameter and 1.5 m long, with a steam inlet, bleed-off valves, and heaters. The vessel is charged with equilibrated collets, sealed, and heated. Degree of puffing varies according to temperature (260 to 427°C) and pressure (7.03 to 14.06 tonnes/m^2). The pressure is suddenly released and the material explodes from the gun. Five to seven minutes may be required to complete the process. The expansion varies from 10 to 16 times.[11]

Products that require less expansion are generally oven puffed. These breakfast foods are almost exclusively made from rice, but sometimes also from corn or mixtures of the two.[85] Puffing is achieved by exposing the product to radiant heat on a belt or by tumbling it in a rotating cylinder. This process produces a three- to fourfold expansion. The oven puffing operation requires the correct balance of moisture content and oven temperature to achieve the desired puffing. The cycle usually takes 90 s.

4. Extruded Snacks

Extruded snacks are a growing segment of the corn-based snack market. In 1998, there were $811 million sales and 127 million kg sold.[3] Corn meal or grits are processed through extrusion cooking and puffing to produce corn curls, puffs, and balls (Figure 11.10). The shape of the puffed extrudate is determined by the die, operational temperature, cut-off knife speed, and other factors. Extrudate

expansion is closely related to product texture and is affected by the viscoelastic nature of the material and the amount of moisture in the material flowing through the extruder die assembly.[92] The puffing of the extrudate is due to the sudden vaporization of water and its escape from the extrudate into the atmosphere.[91] The extrudates are baked or fried, flavored, and packaged to produce the final ready-to-eat product. Mouth-feel is affected by oil content of the products.

Collets, or half products, can be produced by conventional methods (i.e., macaroni presses) or by using a combination of two extruders (Figure 11.10). The first extruder cooks and puffs the raw ingredients while the second cools, forms, and sizes the extrudate into dense collets, which are dried, stored, and then baked or fried into the final product, called third-generation snacks. Baked collets are crisp with a light, crunchy texture. Deep-fat fried collets are crisp with smoother texture due to the uptake of the oil. Preextrusion moisture content of the meal is critical in determining the characteristics and texture of the product. As the moisture content is increased, extrusion cooking temperatures generally drop, and a dense, less expanded product is obtained. High-moisture meals produce hard, dense extrudates that are generally fried. Meals with low moisture produce expanded extrudates that are generally baked to form a light-textured puffed snack. Waxy corn (high amylopectin) tends to produce more fragile, less dense extrudates. Most snacks are packaged at a moisture content of less than 2%. An excellent review of the various aspects of extrusion cooking is given by Mercier et al.[93]

5. Arepas

Approximately 700,000 tonnes of corn are processed annually into arepa flour in nine plants in Venezuela.[22] In 1982, yearly sales of arepa flour was estimated at 482,500 tonnes, with more than 30 kg produced per person.[22] Hard corns with reduced levels of damaged and stress-cracked kernels are absolutely necessary for this type of product. The grain must have excellent dry milling potential.

Large, state-of-the-art-plants commercially produce instant arepa flours that require only hot water to produce the dough. Maize grits from dry milling are cooked and then passed through flaking rolls to gelatinize the starch (Figure 11.11). The flakes are dried and ground into flour with acceptable granulation. With precooked corn flour, arepas can be prepared in approximately 30 min instead of 12 to 24 h required by the traditional process. An alternative method for production of instant arepa flour by extrusion cooking of moistened grits followed by drying, grinding, sieving, and packaging was developed[33]; it is not used, however.

C. Corn Meal

Corn meal has a smaller granulation than grits (1.19 to 0.193 mm; US sieves No. 14 to 75). It is a popular dry corn product because of its long shelf life, freedom from black specks, and bright color. Corn meal is often enriched with thiamin, riboflavin, niacin, and iron. It is used to produce an assortment of chemically leavened baked and fried products such as corn bread, muffins, pancakes, cornsticks, fritters, hush puppies, and spoon bread.

Most corn bread formulations contain wheat flour, chemical leavening agents (i.e., sodium bicarbonate, monocalcium phosphate, etc.), sugar, salt, milk powder, and other ingredients. Wheat flour is included to produce a more aerated, lighter product. Hush puppies are produced from a chemically leavened dough that contains corn meal, wheat flour, eggs, milk, salt, onions, and tomato. Pieces of dough are deep-fat fried for 2 to 3 min. Formulations for various kinds of corn bread are reported by Miller[94] and Sultan.[95]

Corn meal has been used as a vehicle for protein fortification. The meal has been traditionally blended with soy flour and grits or dry milk to enhance protein quality and obtain low-cost protein foods for school lunch programs. The product called CSM has excellent acceptance and is ideally suitable for feeding in school lunch programs.[68]

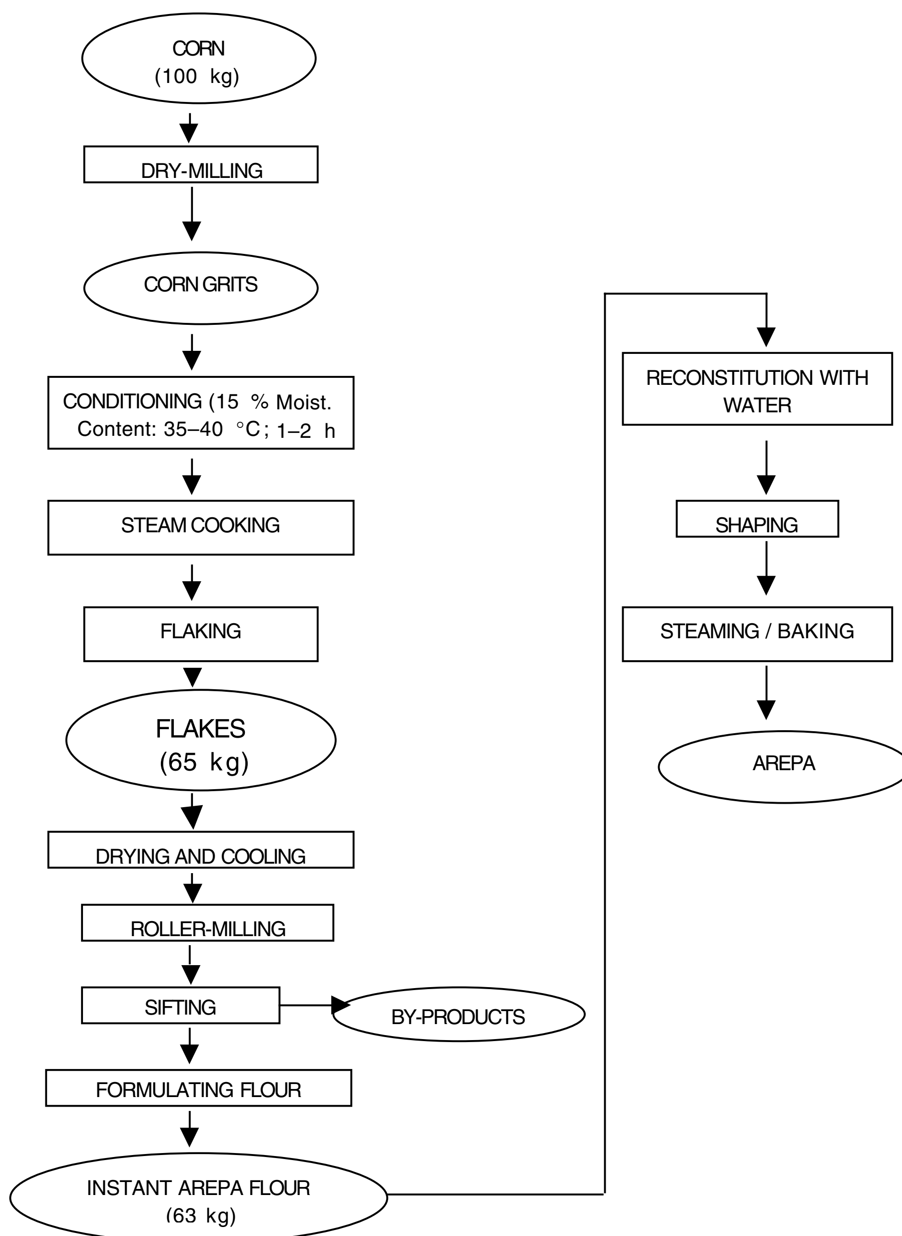


FIGURE 11.11 Flowchart of the industrial process for production of instant arepa flour.

D. Corn Flour

Corn flour consists of fine endosperm particles of less than 0.193 mm (US sieve No. 75). It is widely used as an ingredient in many formulations for pancakes, muffins, doughnuts, breadings, and batters. Corn flour is used extensively in ready-to-eat breakfast foods, snacks, and as a binder in processed meats. The modification and use of corn flour to produce breadings for frozen products that retain crisp texture after microwaving is being investigated.[12]

VII. FOOD CORN QUALITY EVALUATION

The quality of corn for use in food processing varies depending upon the type of processes used. Dry milling, alkaline cooking, snack, and breakfast food production require nearly the same desirable attributes. However, wet milling and alcohol fermentation require corn with significantly different attributes. In fact, a corn that has outstanding quality for dry milling will be unsuitable for wet milling into starch and sweeteners. Wet milling characteristics are covered in Chapter 2. In general, wet millers want soft, high-starch corns that take up water quickly, release the starch readily, and produce the most pure starch and germ, which is processed into oil. Both industries require viable, whole unbroken kernels that have not been dried too rapidly.

"Food corn quality" in this chapter means the quality attributes of corn for dry milling and alkaline cooking or nixtamalization. The attributes are generally similar. Corn for dry milling and nixtamalization should be hard, medium to large kernels, have high test weight, high density, and a pericarp that is easily removed during processing.[96,97] Soft, floury kernels are broken during dry milling and produce low yields of large grits. Soft kernels cook more easily during alkaline cooking, but tend to be overcooked, with high dry matter losses.[96,97] The hard corns require longer to cook but have more tolerance to overcooking and agitation during cooking. There is an optimum hardness for corn used in alkaline-cooking. For example, flint corns do not make good alkaline cooked products because they require too long to cook. In some processes, soft corns are used to make tortillas and chips, i.e., blue corn chips. However, great care must be used to cook and handle the corn. Even then dry matter losses are high; forming and sheeting are often difficult. Commercial hybrid corn company breeders have made excellent progress in selecting for harder food corns. Currently, we may have some hybrids that are too hard for alkaline cooking, but they are fine for dry milling.

The color of corn grain and the cob is critical for alkaline cooking. Kernels with a clean white or bright medium yellow color are most desired. A white cob is preferred. The white cob provides grain with the cleanest, brightest color.[46] Red cob yellow hybrids are sometimes used in the U.S. because the hybrids have otherwise desirable characteristics; white cob hybrids with equivalent yields are not available. The red pigments present in the plant sometimes produce red streaking on the pericarp, which turns brown during alkaline cooking and gives a dirty off-white or yellow appearance to chips and tortillas. Corns with dark yellow color are undesirable because they produce very dark products. For dry milling, the color is not critical.

Pericarp removal during alkaline cooking is affected by both environment and genotype.[98] In some hybrids the pericarp comes off very easily, while in other hybrids it adheres strongly to the kernel. The pericarp of corn is generally removed for production of chips because pieces of the pericarp cause problems during sheeting and cutting of the masa. For table tortillas, the pericarp is only partially removed because the alkaline-treated pericarp forms a gum that binds water and improves the texture of the tortillas. This is an important difference that is often misunderstood.

The density of the corn kernel and the bulk density are usually closely related. We prefer to determine density with a nitrogen pycnometer, which gives readings with accuracy to the third decimal. A density of 1.3 g/cm^3 or higher indicates a kernel with a high proportion of hard endosperm that will likely have good food corn quality. Density is affected by variety and environment. Density generally decreases with early corn hybrids.

The bulk density or test weight of a good corn should be around 74 kg/hl (60 lb/bu) or more. For dry milling, higher test weights within a variety indicate better milling properties provided stress cracks are minimal. Test weight is an excellent index of corn quality provided it is used on known varieties with similar kernel attributes. It is used to pay premiums to farmers who produce specific hybrids for dry milling. It is quick and efficient. Likewise, moisture content and other kernel quality factors should be standardized.

Broken and cracked kernels affect corn quality significantly. Chipped or broken grains allow rapid entry of water, heat, and alkali into the kernel, which results in overcooked kernels that

disintegrate during subsequent operations, causing large dry matter losses and poor quality masa. Stress cracks are not as important in alkaline cooking as for dry milling. However, stress cracks may cause a problem in alkaline cooking when procedures using extensive pumping and rigorous handling are used.[50,97]

The kernel texture of corn is related to the proportion of hard or flinty to soft or floury endosperm present in the kernel. This is often referred to as hard or soft starch by industry. The appearance of a kernel with good quality is relatively easily discerned by examining ears of corn. The kernels with a rounded crown and a very shallow, smooth dent are desirable for alkaline cooking and dry milling. Rating kernel appearance on a 1 to 5 scale based on known standards is a quick and efficient method of selecting for food corn quality in a breeding program. These kernel attributes are related to hardness measurements, kernel density, and test weight.

VIII. FOOD CORN IMPROVEMENT

White food corn improvement programs jointly sponsored by Quaker Oats, Inc. and the American Corn Millers Federation were initiated in 1977. The Snack Food Association sponsored similar research on alkaline cooking and yellow food corn hybrid development from 1984 to 1996. These programs provided modest supplementary research funds to several State Agricultural Experiment Stations to encourage white and yellow food corn hybrid development. Breeding for yield, agronomics, and quality of white corn was greatly expanded by this far-sighted approach. Today, white corn hybrids with markedly better yields, adaptability, and improved quality are coming from the project.[48,99,100] Commercial hybrid seed corn companies have used the germplasm and information generated by these projects to release better adapted white and yellow food hybrids. The yield difference between white and yellow hybrids has been significantly reduced. Yellow food corn hybrids adapted to the Corn Belt have enhanced the efficiency of dry and alkaline processing.

IX. GREEN CORN QUALITY

Internationally, the use of green corn is a very important part of food consumption patterns in corn producing areas. In nearly all countries, except the U.S., field corn is preferred for use as green corn. The ears are harvested when they are in the hard dough stage, boiled in water, parched, grilled or baked in coals, and eaten with various condiments depending upon the particular culture. Butter, various oils, peppers, salt, cream, and many spices are consumed. Cobs of corn sold by street vendors are very popular. Sometimes the corn is removed from the cob, ground, and made into porridges, soups, and various baked products. In Colombia and Venezuela, special kinds of arepas called "cachapas" have a distinctive outstanding flavor because they are made from fresh corn.

Green corn quality varies; certain varieties are preferred by consumers. The texture of the corn is important, with floury or soft endosperm preferred in general over hard endosperm corns.[101] The texture and flavor are important components, but green corn quality has not been defined. Sweet corn used extensively in the U.S. is not preferred probably because it does not produce sufficient yields. The availability and excellent flavor of green corn is preferred worldwide. More information is required to determine the essential parameters affecting green corn quality and how to select for it in breeding programs.

X. SPECIALTY CORNS

The Cornnuts™ company has developed a special hybrid derived from the Cuzco corns of Peru. The kernels are very large with a very soft floury endosperm. This corn is used to produce Cornnuts, which is a popular snack. The large corn kernels are cooked in alkali to remove the pericarp. Then the corn is dried, tempered, deep-fat fried, and flavored to produce the snack.

The development of high lysine, tryptophan corn hybrids (quality protein maize) could result in the production of snacks and other products with increased nutritional value. Modified high lysine corn hybrids have been successfully produced commercially in Brazil[83] and South Africa.[102–104] These hybrids have hard endosperm with increased levels of tryptophan and lysine, which greatly enhance nutritional value. The marketing of foods could be enhanced by use of these products.

XI. MARKETING OF CORN

Both genetics and environment affect the quality, composition, and physical properties of the corn kernel. Grain quality is also greatly affected by harvesting, grain handling, and storage practices.

The importance of food corn quality for alkaline cooking has encouraged the development of companies that specialize in securing corn with acceptable quality. The special corns are cleaned, stored, blended, and sold to different segments of the breakfast and snack industries. Many of these companies contract with corn growers to produce certain food hybrids that have desirable attributes. The type of harvesting, drying, and handling is specified. Food corn producers usually use rotary combines during threshing. Aflatoxin levels are specified and carefully monitored during harvesting and handling in the elevators. The corn, once it is accepted by the food corn company, is recleaned to remove small, broken kernels and any foreign material. Drying of corn is carefully done to avoid stress cracking.

A good food corn supplier tries to provide corn of consistent cooking quality year round to his customers. Many snack food manufacturers rely on food corn suppliers and pay the price to secure a reliable source of corn with consistent quality. Some buy cheap corn and end up paying more in terms of lost dry matter, inconsistent quality of products, and lost manufacturing time. In addition, they run the risk of obtaining corn with unacceptable aflatoxin content. Communication between processors and suppliers is critically important.

XII. BREEDING CORN FOR IMPROVED QUALITY

A. Food Grade White and Yellow Corns

The need and benefit from improved white and yellow corns is great. Bockholt and Rooney[47] and Rooney et al.[48] have suggested selection criteria guidelines for corns in early generation and early yield trials (Table 11.3). For yellow and white corns destined to be used by the dry milling and alkaline cooking industries, kernel hardness, density, bulk density, shape, and color of the kernel and cob are key characteristics considered.

B. Special Corns

Waxy, high amylose, sweet, high oil, blue, red, QPM, and high protein corns produced through ordinary breeding have been incorporated into many different products with improved functionality or nutritional value. For example, 10 to 15% waxy corn cooked with nonwaxy improves significantly the texture of table tortillas and baked tortilla chips; extruded products with greater expansion and lighter texture are produced with waxy corn. High amylose can be used to produce quantities of resistant starch as a source of dietary fiber. Amylose enhances the strength of extrudates. The improved tryptophan and lysine content of QPM hybrids could lead to significant improvement in the nutritional well-being of Mexican people who consume large quantities of tortillas.

C. Genetically Modified Organisms and Corn Quality for Foods

A large number of yellow corn hybrids in the U.S. currently contain non-corn genes which have been inserted to improve the corn hybrid. For example, Bt corn has greater tolerance to corn

TABLE 11.3
Selection Criteria for Food Grade Corns

Early generation
- Use subjective evaluations
  - Kernel texture,% hard endosperm, light box
  - Depth of dent, shallow
  - Kernel shape, spherical crown
  - Color
  - Pericarp removal, alkali cooking
- Early yield trials
  - Measure kernel characteristics
    - Density, pycnometer
    - Hardness, milling and sieving
    - Pericarp removal, alkali cooking
  - Advanced yield trials
    - Hardness
    - Density
    - Micromilling
    - Microcooking
  - Drill strip trials
    - Laboratory trials
    - Plant scale trials

Source: Bockholt and Rooney[47] and Rooney et al.[48] With permission.

earworms, which improves food processing quality because the lack of ear worms reduces the level of damaged kernels; less mycotoxins are formed. Nutrient dense corn hybrids containing genetically modified organisms (GMOs) are nearing commercial production. These developments could revolutionize corn utilization and will be necessary to feed the world's population in the future.

Currently, Europe and other countries have concerns about the safety of GMOs. Many food companies are labelling or avoiding sales of foods containing GMOs. Some grain companies are selling corn that is certified GMO-free. Issues concerning how to measure GMOs efficiently and what constitutes significant GMO contamination are currently under intense debate. The GMO issue is complex, involving trade and political concerns along with human health. We hope that reason will eventually triumph in this debate.

New corn hybrids with value added through breeding are distinctly possible. The new corns will have improved processing properties for dry and alkaline cooking. Other hybrids will be soft for use in wet milling to produce sweeteners, starches, and alcohol. The combination of economics, practical plant breeding, and new technologies make the future for corn improvement bright.

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12 Pipe Corn, Basis of the "Barnyard Briar"

Larry L. Darrah

CONTENTS

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II. Cob Production and Processing
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I. INTRODUCTION

The need for a type of corn (*Zea mays* L.) especially suited for the production of corn cob pipes originated with the establishment of a pipe manufacturing business by Henry Tibbe in Washington, Missouri in 1869.[1] Initial germplasm was obtained from the intermating of different sources of white, open-pollinated varieties which had large, woody cobs that were used for the pipe bowl. Early pipe corn was faulted for having cobs that were "too small, and they are like a sponge, not hard enough, burn out too easily."[2] Improved hybrids from the open-pollinated pipe corn were cooperatively developed in the mid-1960s by M. S. Zuber, located at the Missouri Agricultural Experiment Station with the U.S. Department of Agriculture. At the height of production, corn cob pipes were manufactured by three companies, all located in Washington, MO: Buescher's Industries, Hirschl & Bendheim, and Missouri Meerschaum, only the latter of which remains in business.

Cob pipe production peaked in the 1960s and 1970s, when up to 25 million pipes were manufactured annually. In 1964, a substantial increase in demand occurred when the U.S. Surgeon General linked cigarette smoking with increased health risks. Approximately 2 million pipes are made annually today. Dollar sales in 1975 amounted to approximately $25 million. During this period, one manufacturing company had over 800 ha of pipe corn production. Current production varies from 90 to 140 ha annually. A premium of approximately 20% was paid for raising pipe corn, but the grain yield was expected to be 15 to 20% less than comparable white corn hybrids. Corn cob pipes have sold from $0.29 to over $20 each for wholly hand-made show pipes. The majority of corn cob pipes sell today for less than $3.

Corn cobs suitable for pipe manufacture have a large diameter, preferably over 38 mm, and a length sufficient for at least two 51 mm bowls (Figures 12.1 and 12.2). Tapering from cob butt to tip should be minimal. The cob itself must be woody and have only a small amount of soft, white pithy core. The woody pith should be hard enough so the tobacco fire does not burn the pipe bowl in addition to its contents. After shelling, the large, white pipe corn grain is sold to dry corn millers.

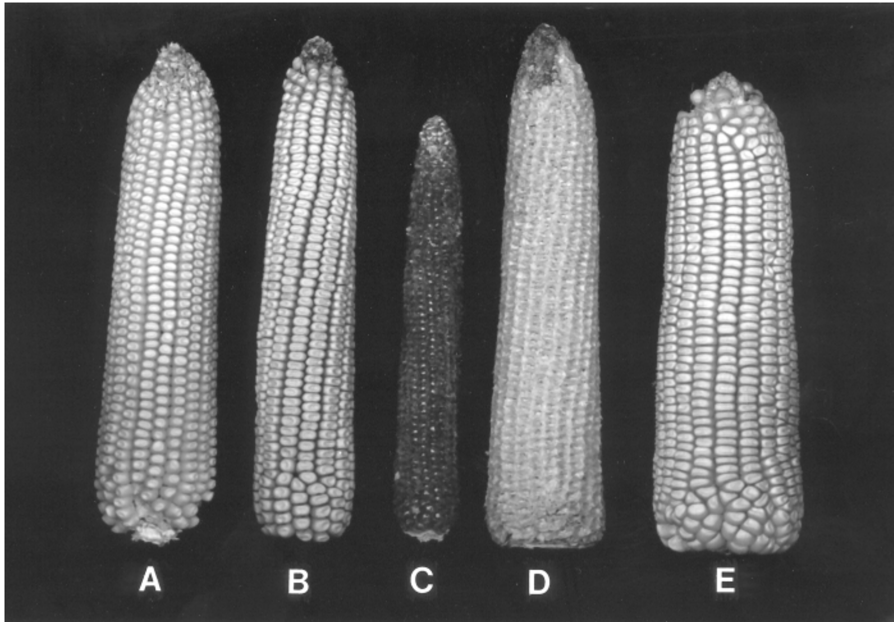


FIGURE 12.1 Contrast of sizes of pipe corn vs. dent corn ears and cobs: **A**, large ear of white food corn (25 cm × 5.5 cm dia.); **B**, typical yellow feed corn ear (26 cm × 4.7 cm dia.); **C**, typical cob of yellow feed corn (22 cm × 2.6 cm dia.); **D**, highly desirable cob of pipe corn (27 cm × 5.0 cm dia.); and **E**, typical Missouri open-pollinated pipe corn (non-hybrid) ear (24 cm × 6.9 cm dia.).

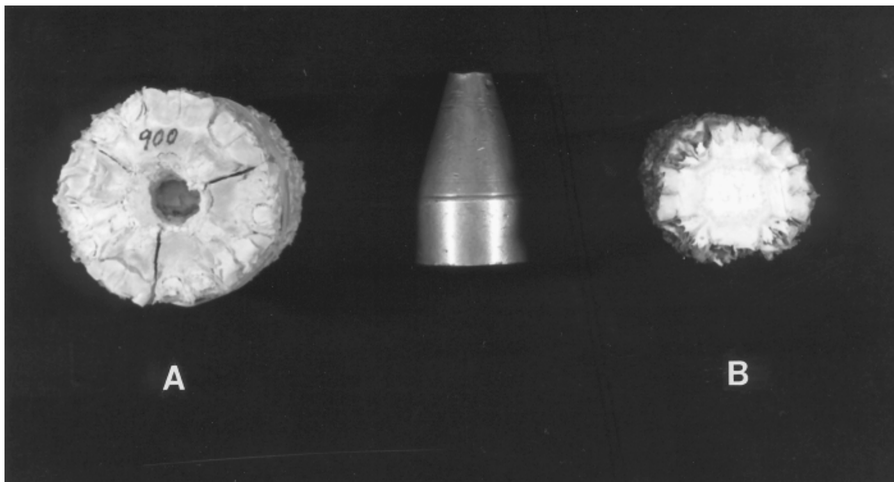


FIGURE 12.2 Cob sections of pipe corn (**A**, 41 mm dia.) and yellow feed corn (**B**, 28 mm dia.) cut for measuring splitting resistance or for pipe bowl manufacture. The experimental pipe cob section shown required 900 load-kg to split in contrast to early hybrids that required 590 load-kg. Between the two sections is the metal cone used to measure splitting resistance.

II. COB PRODUCTION AND PROCESSING

Superior cob development requires high yield per plant. Woodiness of the cob does not develop on plants that are under stress or that do not produce near optimum grain yields. Plant densities of 30,000 ha^{-1} (12,140 acre^{-1}), or less, are used to enhance individual plant development. Higher plant

densities reduce yield per plant and cob quality as measured by cob size, density, and resistance to splitting.[3] In addition, because of the large ear produced high on the plant, growers can minimize lodging by using lower plant densities. Early, open-pollinated varieties tended to root and stalk lodge easily. Commercial pipe corn grain yields over 6200 kg ha^{-1} have been obtained in the Missouri river bottom production areas.[1] Experimental hybrid plot yields on irrigated upland soils near Columbia, MO, have ranged up to 8500 kg ha^{-1} and up to 4600 kg ha^{-1} on dry land.

Most cob production occurs on Missouri river-bottom land near the site of manufacturing in Washington, MO. During the peak period of production, however, sites as far away as Malta Bend, MO (256 km from Washington, MO), have been used, but still located on high potential, river-bottom soil to which these genotypes are adapted. Fertility levels are similar to those used for normal dent corn grown in the production area. Procedures for control of weed, disease, and insect pests are also similar.

Harvest of the ears is done by an ear-picker rather than a combine picker–sheller. After drying, ears are shelled using special machines similar to those used by commercial seed companies for shelling seed corn. The objective, however, is not to minimize damage to the seed, but to the cob. Cobs are cured under ambient, indoor conditions for up to 2 years. Bowls are manufactured by cutting 51 mm sections from the cob center, drilling a tobacco hole, turning down and smoothing the outside of the cob, plugging the bottom of the tobacco hole with a wood dowel, filling the cob exterior with plaster of Paris, sanding, shellacking, drilling of the stem hole, and fitting of the pipe stem (Figure 12.3).

III. PIPE CORN BREEDING

From the 1900s to the late 1950s, the source of cobs for pipe manufacture was the Missouri Meerschaum open-pollinated pipe corn variety. In 1947, a breeding program for pipe corn improvement was initiated by M. S. Zuber at the Missouri Agricultural Experiment Station in cooperation

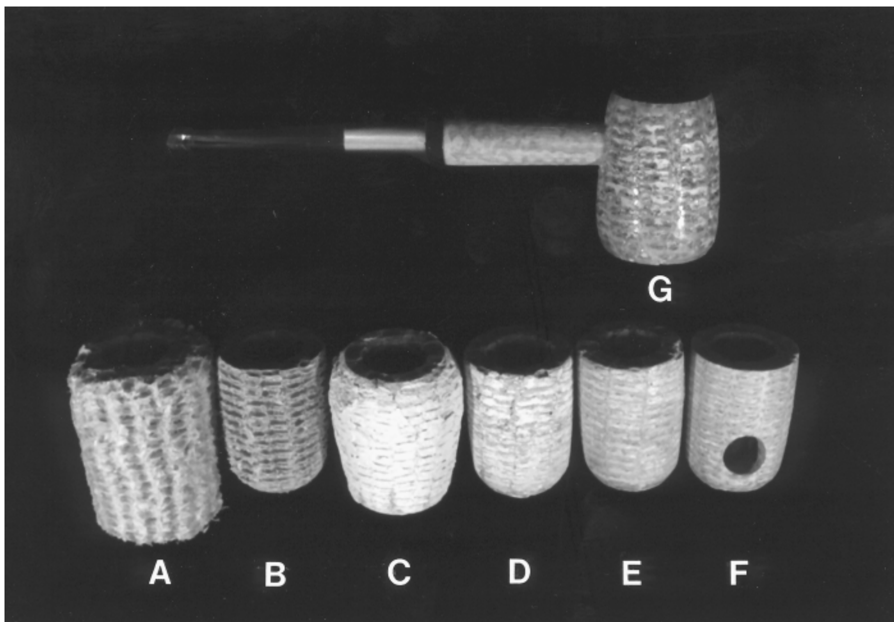


FIGURE 12.3 Stages of corn cob pipe manufacture: **A**, cob section with tobacco hole; **B**, section turned down and smoothed with a lathe into bowl shape; **C**, bowl exterior filled with plaster of Paris; **D**, sanded bowl; **E**, shellac applied; **F**, stem hole drilled; and **G**, finished pipe.

with the U.S. Department of Agriculture. Hybrids were being rapidly accepted and growers were reluctant to continue growing the open-pollinated pipe corn variety. Thus, inbred lines were extracted from the open-pollinated pipe corn, intercrossed, and tested for hybrid performance. Selection for suitable cobs was initially based on sawing a 51 mm section from the cobs being evaluated and crushing it in a hydraulic press. More recently, selections were based on measurement of the force needed to split the woody part of the cob by pressing a brass cone into the soft pith (Figure 12.2). Throughout the selection program, emphasis was placed on shorter plants, lower ears, and increased resistance to root and stalk lodging. Resulting lines were released and commercially used in double-cross hybrid combinations (Mo Pipe 4 and Mo Pipe 12) and later in a three-way cross (Mo Pipe 65).

In 1978, ear-to-row selection was initiated in the Missouri open-pollinated pipe population to more rapidly increase resistance to splitting that was occurring because of automating tobacco hole boring. Three cycles of selection and line extraction were completed. Experimental hybrids evaluated in 1989 had greater cob diameter and showed more than a 200% gain in resistance to splitting compared to the commercially grown hybrid Mo Pipe 65.

Development of an improved double-cross pipe corn hybrid, known as Mo Pipe DX, led to comparison of planting densities and performance as compared to Mo Pipe 65. Irrigated trial data from one location in 1994 showed that at 30,890 plants ha^{-1} (12,500 plants $acre^{-1}$), Mo Pipe DX relative to Mo Pipe 65 had a 17% yield increase, a 9% cob diameter increase, a 61% splitting resistance increase, and a 31% increase in the total number of full-size bowls (Table 12.1; Figures 12.4 and 12.5). Under dryland conditions, these increases were even greater. Excellent pipe cob production with Mo Pipe DX was also observed at the highest density evaluated, 37,060 plants ha^{-1} (15,000 plants $acre^{-1}$).

TABLE 12.1
Comparison of Mo Pipe 65 and Mo Pipe DX at Four Plant Densities Under Irrigated and Dryland Conditions at Columbia, MO

Hybrid	Plant Density (ha^{-1})	Yield (kg ha^{-1})	Cob Diam. (mm)	Splitting Resist. (load-kg)	Full-Size Bowls (No.)
		Irrigated			
Mo Pipe 65	18,530	4136	39.5	206	56
	24,710	5009	41.0	176	72
	30,890	6170	41.0	200	81
	37,060	6942	40.0	162	102
Mo Pipe DX	18,530	4676	46.2	346	70
	24,710	6270	45.4	364	88
	30,890	7212	44.9	322	106
	37,060	8586	44.4	336	121
LSD 0.05		885	2.6	80	12
CV%		8	4	17	8
		Dryland			
Mo Pipe 65	18,530	2529	39.0	157	37
	24,710	2580	36.9	181	38
	30,890	2850	35.4	125	40
	37,060	2467	36.7	162	32
Mo Pipe DX	18,530	3151	44.9	327	47
	24,710	4337	42.8	295	61
	30,890	4632	42.1	308	60
	37,060	4155	41.3	278	50
LSD 0.05		979	2.6	107	15
CV%		17	4	26	19

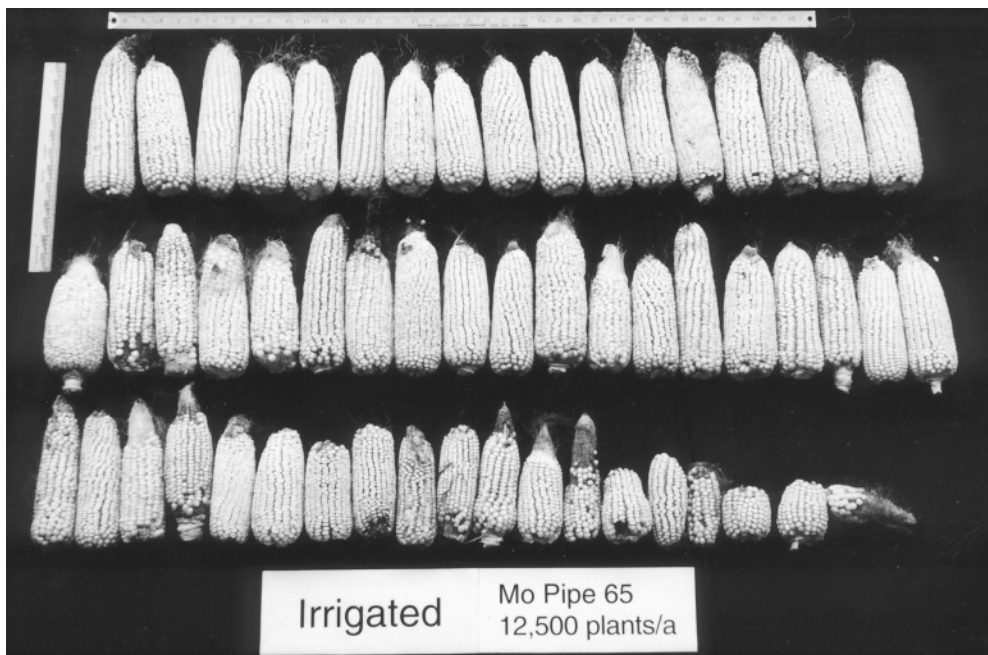


FIGURE 12.4 Mo Pipe 65 ears harvested from an irrigated plot grown at 30,880 plants ha^{-1} (12,500 plants $acre^{-1}$).

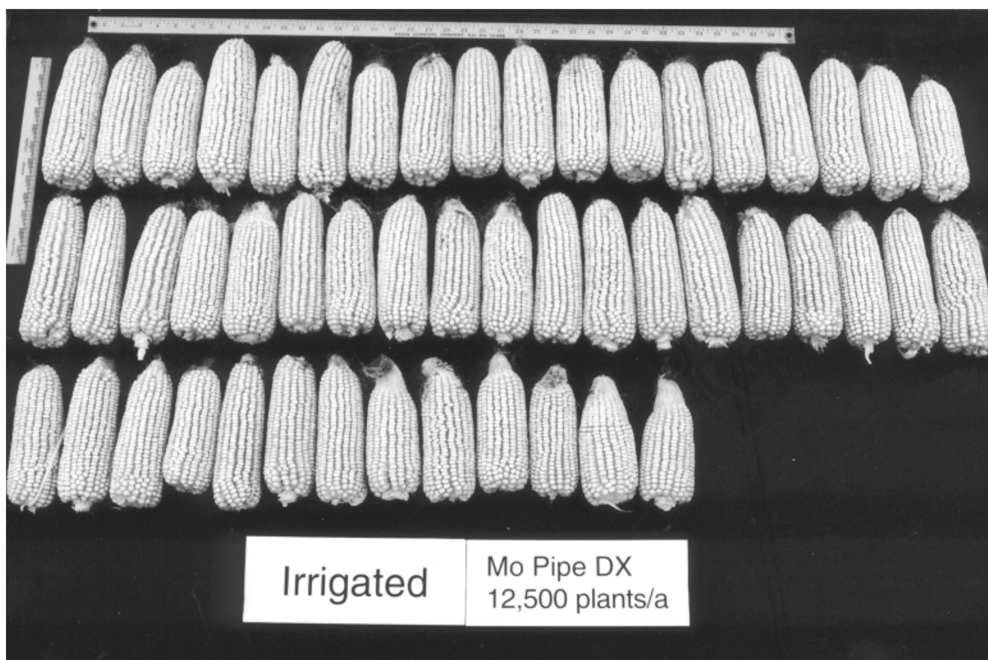


FIGURE 12.5 Mo Pipe DX ears harvested from an irrigated plot grown at 30,880 plants ha^{-1} (12,500 plants $acre^{-1}$).

IV. PIPE CORN GERMPLASM

Two accessions representing the original open-pollinated pipe corn variety are held at the North Central Regional Plant Introduction Station in Ames, Iowa, under plant introduction (PI) numbers PI221884, Pipe Corn and PI 221888, Missouri Pipe Corn Meerschaum. Eight pipe corn inbred lines have been jointly released since 1960 by the Missouri Agricultural Experiment Station and U.S. Department of Agriculture (Table 12.2). Experimental lines used in Mo Pipe DX are available from the Missouri Agricultural Experiment Station.

Commercial pipe corn production was changed over from the open-pollinated variety to hybrid pipe corn beginning in the early 1960s with Mo Pipe 4 (Table 12.3). Mo Pipe 12 was released for use in 1963 and Mo Pipe 65 was released in 1976. Mo Pipe DX has been grown as a significant part of the total production since about 1994. Some of the open-pollinated variety may be grown on very limited hectarage at very low density in order to produce exceptionally large cobs for show-type, hand-made pipes.

TABLE 12.2
Pipe Corn Inbred Lines Jointly Released by the Missouri Agricultural Experiment Station and U.S. Department of Agriculture

					Color[a]		
Inbred	Maturity[b]	DF[c]	Source	Year Released	Grn.	Cob	Breeder
Mo8W	—	77	Open-pollinated Pipe Corn	1960	W	W	M. S. Zuber
Mo9W	—	76	Open-pollinated Pipe Corn	1960	W	W	M. S. Zuber
K10[d]	—	71	Pride of Saline	1960	W	W	M. S. Zuber
Mo15W	—	74	Open-pollinated Pipe Corn	1963	W	W	M. S. Zuber
Mo16W	—	75	Open-pollinated Pipe Corn	1963	W	W	M. S. Zuber
Mo23W	900–1000	—	(K10 × Ky49/Mammoth White Pearl)	1976	W	W	M. S. Zuber
Mo24W	900–1000	—	(K10 × Ky49/Ziler Hi Cob)	1976	W	R	M. S. Zuber
Mo25W	900–1000	—	(K10 × Ky49/Ziler Hi Cob)	1976	W	W	M. S. Zuber
MOPP-132-2–1	1000	—	Open-pollinated Pipe Corn	1993[e]	W	W	L. L. Darrah
MOPP-132-2-3	1000	—	Open-pollinated Pipe Corn	1993[e]	W	W	L. L. Darrah

[a] W represents a white color and R represents red. Grn. refers to grain.
[b] Agricultural Experiment Station maturity rating in which 100 is earliest and 1200 is very late.
[c] Days to flowering.
[d] Joint release with Kansas Agricultural Experiment Station, Manhattan, KS.
[e] Unreleased Agricultural Experiment Station line used in Mo Pipe DX.

TABLE 12.3
Pedigrees of Commercially Important Pipe Corn Hybrids

Hybrid	Pedigree	Year released
Mo Pipe 4	(Mo8W × Mo9W)(K10 × Ky49)	~1960
Mo Pipe 12	(Mo15W × Mo16W)(Mo8W × Mo9W)	1963
Mo Pipe 65	(Mo15W × Mo16W)Mo24W	1976
Mo Pipe DX	(Mo15W × Mo16W)(MOPP-132-2-1 × MOPP-132-2-3)	1993

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13 Silage Corn

James G. Coors and Joseph G. Lauer

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I. INTRODUCTION

Farmers have been preserving forages by ensiling them for several thousand years. The term silo is derived from the Greek word "*siros*" meaning pit or hole in the ground.[1] The Egyptians were familiar with ensiling techniques and recognized the importance of air-tight sealing for forage preservation, as depicted in paintings dating 1000 to 1500 B.C.[2] Native Americans of the southern U.S. were also known to preserve their stores of corn (*Zea mays* ssp. *mays*) in pits in the ground.[3] Procedures of preserving forages have now evolved to the point where it is known that there are at least three characteristics of plant materials necessary to ensure good silage[4]: (1) adequate level of fermentable substrate, (2) a relatively low buffering capacity, and (3) a dry matter concentration of greater than 200 g/kg. These characteristics in combination with anaerobic storage conditions promote effective fermentation. Anaerobic conditions are needed to reduce the activity of respiratory enzymes in plant material. Such enzymes tend to promote heat build up and reduce both total dry

matter and nutritional value of silages if left unchecked. There is also competition between lactic acid-producing and lactic acid-utilizing bacteria in the silo. Lactic acid-producing microbes are facultative anaerobes that ferment sugars (mainly glucose and fructose) to produce lactic acid. If the lactic acid-producing bacteria prevail, the silo pH will be ideally lowered to 4.0 over a period of several days, and plant material will be well preserved. Anaerobic and acidic conditions discourage lactic acid-utilizing microbes, such as *Clostridia* bacteria, that degrade amino acids to products of poor nutritive value, but the higher the moisture content of the silage, the lower the pH *Clostridia* can remain active.[4]

Corn has several practical attributes that contribute to its potential as a silage. Corn is a versatile crop that can be planted from early to late spring. Farmers have the option of harvesting corn for either forage or grain. Corn has high dry matter yields that are obtained in a single harvest, and harvests can be completed prior to significant leaf loss. Corn silages are characterized by stable fermentation due to dry matter concentrations greater than 200 g/kg, high nonstructural carbohydrate concentration, and low buffering capacity.[4,5] Compared with other forages, corn silage has relatively high fiber concentrations, as measured by neutral or acid detergent fiber (NDF and ADF, respectively), but low lignin concentrations, leading to low lignin/NDF or lignin/ADF ratios.[6] In contrast to most other forages, corn has consistent nutritive value over a longer harvest period. As the highly digestible grain develops, it compensates for the decline in quality of the stover fraction of the plant. Well managed, high dry matter corn silages can have relatively high energy and high intake when compared with alfalfa and other grasses. The main nutritional deficiency of corn silage is low protein concentration (Table 13.1).[7]

Ensiling corn may produce some nutritive losses typically affecting both total dry matter and nitrogen.[8] Total energy losses are reported to range from 7 to 40%.[4,8] Forage resulting from a lactic acid fermentation is characterized by conversion of simple sugars to organic acids, and protein nitrogen is degraded to non-protein forms (mostly amino acids). Most fiber components remain intact, although there may be some acid-hydrolysis of hemicellulose.[9] These changes in simple carbohydrate, protein, and fiber composition are associated with reduced intake and digestibility.[4] Intake and digestibility have been reported to decrease by 5 to 30% and 2 to 4%, respectively.[10,11] Fortunately for plant breeders, interactions involving ensiling methods and corn genotypes for whole-plant quality have not been shown to be significant,[9] indicating that selection for nutritive value on the basis of non-ensiled whole plants would not be confounded by subsequent storage conditions.

Fermentation losses can be restricted somewhat by ensiling higher dry matter, more mature silages (300 to 350 g/kg). More mature silages have a greater percentage grain, and starch is less susceptible to fermentation than sugars.[4,5] Fermentation of water-soluble carbohydrates during ensiling leads to increased concentrations of ADF and acid detergent lignin (ADL) and lower concentration of digestible organic matter after fermentation.[10,11] Dairy cattle may be particularly sensitive to fermentive effects associated with ensiling high-moisture forages, and intake and milk production may be reduced.[12]

Silage making is an especially attractive option for preserving forages in northern temperate regions with restricted growing seasons. Grasses have been ensiled in Sweden and the Baltic countries since early to mid-1800s.[1,2] Grass silages were produced in Hungary starting around 1860, and the practice quickly spread to Germany.[2] A French farmer, Auguste Goffart, published the first book dealing with ensilage in 1877 based primarily on experiences with corn,[13] and he was christened "the father of modern silage."[1] In England prior to 1880, silages were preserved in simple pits or stacks. After 1880, Goffart's contributions were reflected in the construction of many brick silos.[2] The first silo built in the U.S was erected by Francis Morris of Maryland in 1876.[1] The U.S. edition of Goffart's book was published in 1879, and by 1900 there were approximately 100,000 or more silos, mostly towers, in the U.S.[2]

Current use of corn as an ensiled forage is widespread in northern temperate regions, particularly in Europe and Canada. Nearly 4×10^6 ha of corn silage are grown in Europe with France and

TABLE 13.1
Carbohydrate[a] and Protein[b] Fractions in Common U.S. Feeds

Feedstuff	NDF (% of DM)	Lignin (% of NDF)	NSP (% of DM)	Starch (% of NSC)	CP (% of DM)	Soluble N (% of CP)	ADFIP (% of CP)	NPN (% of SP)	NDFIP (% of CP)
Alfalfa hay, north									
Early bloom	42.0	16.9	7	10	19.0	30.0	10.0	96	17.8
Mid bloom	46.0	18.9	7	10	17.0	28.0	14.0	96	25.2
Alfalfa hay, south									
Early bloom	40.0	20.0	7	10	25.0	30.0	10.0	96	17.8
Mid bloom	44.0	22.7	7	10	22.0	28.0	14.0	96	25.2
Alfalfa silage, north									
Early bloom	42.0	16.9	7	10	19.0	50.0	15.0	100	26.7
Mid bloom	46.0	18.9	7	10	17.0	45.0	18.0	100	32.0
Grass hay									
Early bloom	67.0	7.5	1	6	9.1	25.0	6.1	96	31.0
Mature	72.0	12.5	1	6	7.0	25.0	6.5	96	31.0
Corn silage, north									
45% grain	41.0	7.3	—	100	9.0	45.0	7.9	100	16.4
25% grain	52.0	9.6	—	100	8.3	55.0	8.5	100	16.0
Corn silage, south									
45% grain	45.0	9.0	—	100	9.2	45.0	7.9	100	16.4
25% grain	55.0	10.9	—	100	8.1	50.0	8.0	100	16.0

[a] DM = dry matter; NSP = nonstructural polysaccharides (pectin, galactins, fructans, betaglucans, etc.); NSC = nonstructural carbohydrates.

[b] CP = crude protein; NPN = non-protein nitrogen; ADFIP = acid detergent insoluble protein; SP = soluble protein; NDFIP = neutral detergent insoluble protein.

Source: Adapted from Sniffen et al.[7]

Germany having the greatest production (Table 13.2).[14] Canada currently harvests over 190,000 ha of corn silage, and the production is concentrated in Ontario (63%) followed by Quebec (21%).[15] In the U.S. production is primarily in the northern Corn Belt, and the states with largest production are usually Wisconsin, New York, and Minnesota (Table 13.3).[16] U.S. silage production increased from approximately 1.5×10^6 ha in the 1920s to 4.6×10^6 ha in 1976 (Figure 13.1).[16] Since 1976, U.S. production has decreased to 2.3×10^6 ha in 1997. In 1997, percentage of total corn production harvested as silage was 7.2%, but percentage of silage produced, based on either total grain or overall corn production, is extremely variable and reflects the degree of seasonal stress. For example, percentage silage production usually increases to 12% or more in years with moderate to extreme moisture stress (e.g., 1934, 1936, 1964, 1974, 1976, and 1988).

II. NUTRITIVE VALUE OF CORN SILAGE

Concepts of nutritive value for corn silages necessarily reflect ruminant requirements, and essential aspects of quality deal with amount of feed consumed and rate and extent of digestion. For ruminants, feeds are fermented prior to gastric and intestinal digestion. The unique features of ruminal fermentation are that (1) energy in complex carbohydrates is made available in the form of volatile fatty acids that are absorbed through the rumen wall, and (2) dietary nitrogen is initially used to satisfy microbial requirements, and there is less need for exogenous sources of essential amino acids.

Rate of feed degradation in the rumen profoundly affects fermentation end products and animal performance, and the fate of the feed is determined for the most part by relative rates of fermentation and passage. The Cornell Net Carbohydrate and Protein System[7,17,18] includes a model for feed degradation in the rumen, passage of undegraded feed to the lower gut, and amount of energy and protein that is available to the animal. Figure 13.2 depicts the complex interactions involving dietary protein and carbohydrate fractions accounted for in the Cornell model. Protein can be partitioned into at least three fractions: nonprotein nitrogen (NPN), true protein, and bound true protein. Most soluble protein in silages is in the form of NPN that is rapidly converted to ammonia and either utilized for microbial growth or absorbed across the rumen wall. True protein is in the medium and slowly degradable fraction, some of which may escape ruminal degradation and be absorbed in the lower gut. Bound protein is associated with lignin, tannin-protein complexes, and Maillard products resistant to ruminal degradation. Similarly carbohydrates can be classified on the basis of degradability. Sugars and starches are rapidly degradable, the available cell wall is slowly degradable, and the portion of cell wall bound with lignin is unavailable. The ratio of dietary nitrogen to carbohydrate affects the overall nutritive value of rations.[19] If the nitrogen requirement of rumen microbes is not met, feeding of additional carbohydrate may be ineffective and perhaps detrimental. If dietary nitrogen meets or exceeds microbial requirements, inadequate dietary carbohydrate will lead to excess NPN, which is fermented to ammonia, absorbed through the rumen wall, converted to urea by the liver and excreted. Feeding of additional carbohydrate in this situation would promote more efficient fermentation and promote utilization of excess rumen ammonia.

Corn silages are comparatively low in protein, and corn protein is mostly degraded to NPN during ensiling.[7] Feed rations including corn silage usually require some form of nitrogen supplement. The carbohydrate pool has a relatively greater impact on nutritional value of corn silage than does protein. The most significant carbohydrate sources for rumen microbes are starch and the available and bound carbohydrate fractions of the cell wall. Both the amount and digestibility of starch and cell wall carbohydrate affect animal performance. Until recently, starch was assumed to be nearly completely degraded and available to ruminants, and it was not a primary focus of animal nutritionists and plant breeders. More recent evidence indicates that starch digestibility of corn silage is variable due to both genetic differences among hybrids and physiological processes related to kernel maturation, topics that will been covered in more detail other sections of this chapter.

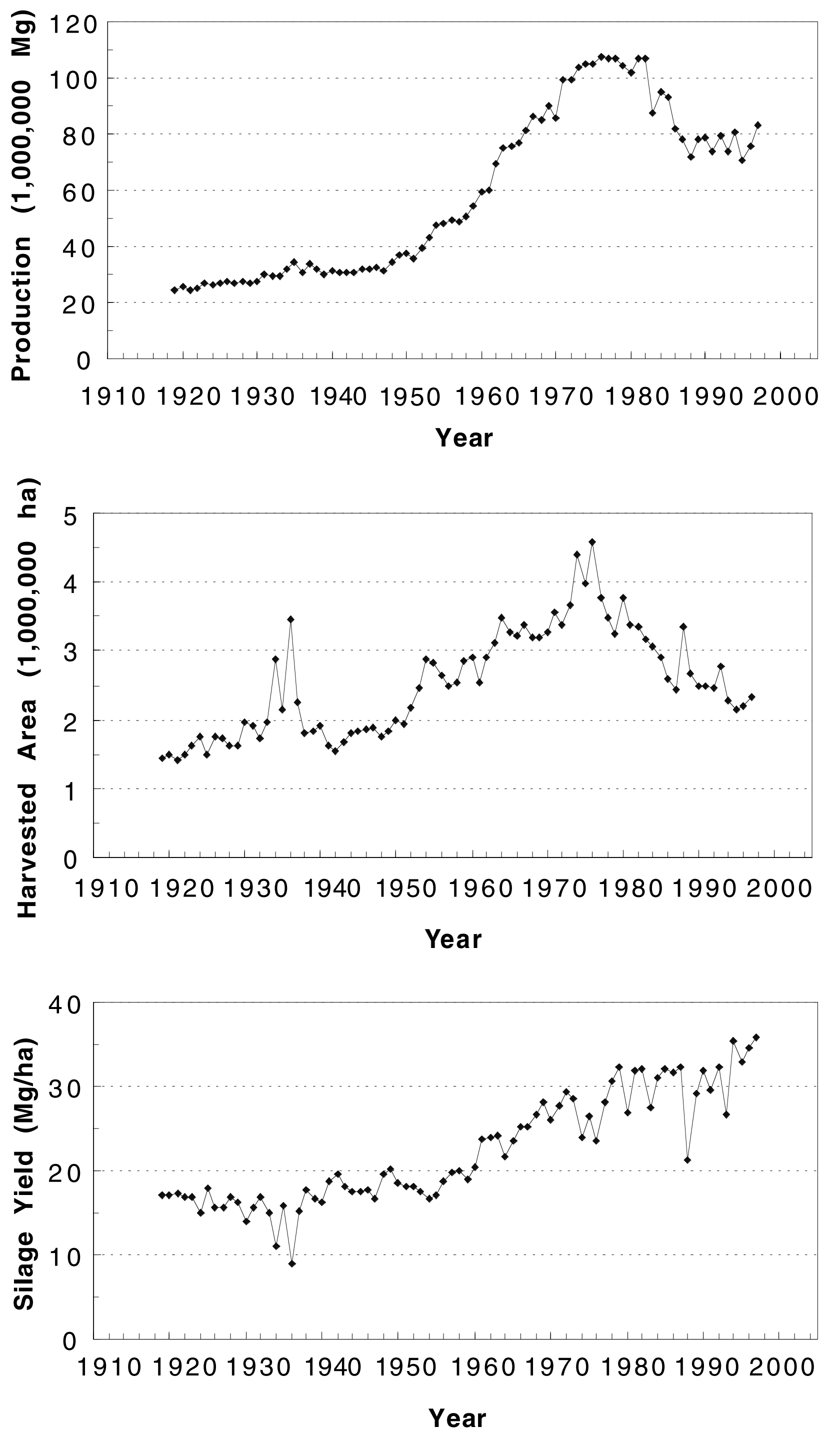


FIGURE 13.1 U.S. corn silage production, harvested area, and yield from 1919 through 1997. (From USDA, Crop Reportin Board, 1997.)

TABLE 13.2
Production Statistics for Corn Silage for 12 European countries in 1997

Country	Harvested Area (1000 ha)	Percentage of Total Corn Area Harvested for Silage	Harvested Production (1000 Mg)	Average Yield (Mg ha^{-1})
Austria	85	47	3918	46
Belgium	173	88	6648	38
Denmark	42	100	1541	37
Germany	1326	78	57657	43
Greece	3	1	—	—
Spain	109	19	5116	47
France	1578	48	52819	33
Italy	302	23	--	--
Luxembourg	10	100	476	48
Netherlands	223	95	8982	40
Portugal	122	40	—	—
United Kingdom	110	100	4162	38

Source: Data from Anon.[14]

TABLE 13.3
Ten U.S. States with the Greatest Corn Silage Production

State	Average Harvested Area from 1988 to 1997(1000 ha)	Percent of Total Corn Area Harvested for Silage from 1988 to 1997
Wisconsin	327	22
New York	218	47
Minnesota	217	8
South Dakota	177	12
Pennsylvania	173	30
Michigan	128	13
Iowa	122	2
North Dakota	111	32
Nebraska	104	3
California	95	56
Total U.S.	2517	8

Source: Data from Anon.[16]

The cell wall includes cellulose, hemicellulose, lignin, and insoluble ash. Most studies on the nutritional composition of corn silage have dealt with three cell wall fractions as determined by the detergent methods of Van Soest[19]: (1) total cell wall constituents (CWC) or NDF; (2) ADF, including primarily cellulose and lignin; and (3) lignin. Feeds high in fiber (NDF or ADF) typically take longer to degrade than less fibrous feeds and, therefore, intake may be reduced. Lignin concentration primarily influences extent of fiber degradation thereby affecting digestibility. Of particular consequence is the amount of cell wall lignification (often expressed as lignin/NDF or lignin/ADF), which increases in most tissues as plants mature and leads to lower digestibility.

Lignin is a complex phenolic-derived macromolecule of various forms arising from the polymerization of three primary precursors (i.e., coniferyl, sinapyl, and *p*-coumaryl alcohols).[20] Extent of cell wall lignification and composition of phenolic units may account for variability in digestibility within and among plant species as well as among distinct plant tissues. In grass leaf blades,

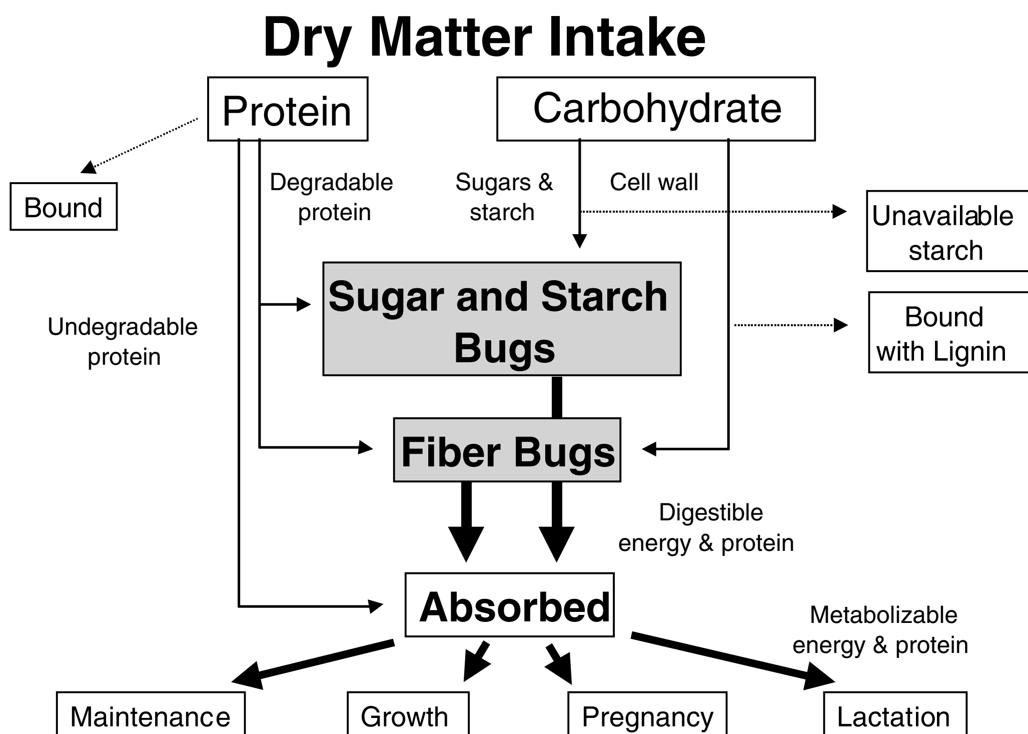


FIGURE 13.2 Model illustrating some of the processes involved with ruminal and postruminal digestion of feedstuffs. (Adapted from Fox, D. G., Department of Animal Science, Cornell University.)

lignocellulosic fibers are characterized by relatively high concentration of cinnamaldehyde units in vascular tissues (xylem cells) and syringyl units in sclerenchyma (mestome sheath). In C4 species such as corn, the parenchyma bundle sheath is also highly lignified. In grass stems, the epidermis, sclerenchyma ring, vascular tissue are usually lignified, and stem parenchyma tissue may become lignified with age.[21]

Mechanisms by which lignin restricts degradation by rumen microbes are poorly understood. Cellulolytic bacteria require physical attachment to fiber particles before degradation occurs,[21–24] and certain tissues, such as the mestome sheath and xylem cells, commonly lack significant bacterial association.[21] Lignin may influence overall extent of fiber digestion by means of physical shielding of cellulose or hemicellulose, or lignin's effect may involve more specific molecular interactions.[25,26] Microbial inhibition by free phenolic acids is probably not the predominant cause of lignin's effect on digestibility,[27,28] but it is quite difficult to assign specific inhibitory effects to individual phenolic acids, since they are usually deposited in coordination with lignin polymerization.[29]

In the past, lignins were subdivided into "core" and "non-core" types.[27] Core lignins were defined as the highly condensed polymeric matrices consisting primarily of guaiacyl and syringyl units derived from coniferyl and sinapyl alcohols that are covalently linked to hemicellulose in the cell wall. Non-core lignins were defined as the low molecular weight phenolic monomers usually covalently linked to either core lignin (e.g., *p*-coumaric acid) or hemicellulose (e.g., ferulic acid). Lower molecular weight phenolic acids, such as *p*-coumaric acid, can impede degradation of bound structural carbohydrate, and free phenolic esters may inhibit rumen microbes.[21,27] The separation of lignin into core and non-core fractions, while convenient for study, may be somewhat artificial. As emphasized by Hatfield et al.,[20] the functional effect of lignin is better described in terms of the extent of ester and ether cross linkages of *p*-coumaric and ferulic acid to lignin macromolecules

and to the arabinose side chains of the xylans. Such linkages determine the tensile strength and impermeability for those cells involved in mechanical support or water transport, such as sclerenchyma and xylem tracheary elements.[24,26,30–33] Hatfield et al.[20] provide an excellent review of cell wall structure, and the reader is encouraged to examine this source for a more complete treatment of this subject.

Analytical methods for determining forage quality have changed considerably over the past several decades.[34] From a nutritionist's perspective, the objective of feeding forages is to provide energy to animals. However, producers must not only consider the quality or energy of the forage that is fed but also the yield of that forage from the land base that he manages. A producer is interested in both yield and quality. The question becomes how do producers, nutritionists, agronomists, and breeders estimate energy (quality) of forages? Although animal feeding trials are the best methods for evaluating silage value, these are expensive and are not practical when evaluating a large number of hybrids and/or agronomic treatments. Therefore, alternative methods to evaluate forages have been developed by many researchers. The practical utility of the more recent procedures now makes it possible to assess forage nutritive value on a broad scale. The 48-h *in vitro* digestion with rumen liquor described by Tilley and Terry[35] provides the basis for many current techniques, especially those used by plant breeders.[36] The two-stage procedure starts with a 48-h period where the forage sample is fermented in a buffered solution of rumen microflora. During this period nearly all soluble carbohydrates are fermented, and a proportion of CWC are degraded, the proportion determined primarily by extent of cell wall lignification. The second stage involves an acid-pepsin solution, simulating intestinal digestion, that hydrolyses rumen microbes as far as possible. The remaining residue contains undegraded cell wall and remnants of rumen microflora. The second stage can be replaced by treatment with neutral detergent as described by Van Soest et al.,[37] and this procedure eliminates most microbial remnants from the residue. Therefore, the remaining fiber reflects the "true" digestible dry matter. By first measuring NDF concentration of the dried forage, then measuring NDF remaining after fermentation, the digestibility of CWC can be determined [often referred to as either cell wall digestibility (CWD) or *in vitro* NDF digestibility (IVNDFD)]. Another variant of the *in vitro* technique involves measuring gas production during *in vitro* fermentation, and this allows measurement of the metabolizable energy content of forages (e.g., Menke et al.[38]). Commercially available cellulases are also used to estimate the degradability of cell wall materials, and they seem to have some promise.[39,40] In France, silage varieties are registered using an energy value prediction model that involves enzymatic estimates of digestibility, along with mineral concentration and crude protein.[41]

Laboratory assessment of quality, particularly if it involves *in vitro* rumen fermentation, is time consuming to the point of being impractical in a breeding program. Near infrared reflectance spectroscopy (NIRS) can be used to increase speed and number of samples assayed. NIRS analysis is based on the reflection of infrared radiation from ground forage samples. A calibration set of samples is analyzed using standard wet-lab analyses, and these data are used to develop a set of calibration equations relating infrared wavelength and intensity to chemical composition. The multivariate regression techniques involved are highly sophisticated, and some diligence is required to ensure that the data provided by NIRS are biologically relevant and have any true predictive utility.[42,43] Care must be taken when estimating complex quality traits involving lignin and digestibilities, although NIRS prediction of extent of *in vitro* rumen fermentation and fungal cellulase digestion is possible.[44,45] The University of Wisconsin Corn Extension program as well as many private laboratories are routinely using broad-based NIRS prediction equations to estimate digestibility of commercial silage hybrids.[46]

III. GERMPLASM AND SOURCE MATERIALS FOR SILAGE CORN

Corn breeders and producers are beginning to question whether the best grain-producing hybrids are also the best silage-producing hybrids. While this issue needs to be addressed on both a yield

and nutritional basis, limited quality information was available until relatively recently. Before reviewing concepts of nutritional quality, a brief examination of historical data and past research will provide some insight on trends in dry matter production.

A. Relationships among Grain Yield, Forage Yield, and Quality

Whole-plant yields of silage corn have increased dramatically since the early part of the twentieth century (Figure 13.1). During this period, whole-plant yield in the U.S. has increased at a linear rate of approximately 0.09 Mg/ha/yr (dry matter basis).[16] Plant breeders have been selecting corn varieties primarily on the basis of grain yield rather than whole-plant yield, so it seems likely that the increase in whole plant yield is mostly due to an increase in the grain fraction. This would tend to increase the harvest index (grain-to-stover ratio), and several studies document such a trend. Russell[47] measured an average harvest index of 44.6% in four pre-1930 open-pollinated varieties, which was lower than the average harvest index of 50.2% for four 1980 era hybrids. Duvick[48] reviewed overall performance of U.S. hybrids from 1930 to 1970, and he reported that the more recent hybrids had higher stover weights and a small but significant increase in harvest index. Tollenaar[49] reported a similar increase in harvest index for Canadian hybrids in use between 1959 and 1988. A study of European corn hybrids bred between 1950 and 1980[50] revealed that for early-maturing germplasm, total dry matter increased at a rate of 0.07 Mg/ha/yr, which was slightly less than the 0.08 Mg/ha/yr measured for grain yield. In later-maturing germplasm total dry matter actually decreased by –0.02 Mg/ha/yr, while grain yield increased by 0.04 Mg/ha/yr. Harvest index evidently increased because in both trials total dry matter had an equal or lower rate of change than the grain component.

Recurrent selection programs for increased grain yield also show indirect effects on forage yield substantiating historical trends toward higher harvest index. At Iowa State University, most recurrent selection programs showing increased grain yield over cycles were also associated with an overall increase in harvest index.[51] Moll and Kamprath[52] found that 10 cycles of full-sib recurrent selection for grain yield substantially increased grain yield from 4.59 Mg/ha to 6.97 Mg/ha without any consequent increase in stover yield, which resulted in a charge of harvest index from 1.08 to 1.34.

Unfortunately, there are no published studies of historical trends in nutritive value of U.S. silage corn. The only available data are from the rare assessments of nutritional composition for corn grain. Duvick and Cassman[53] reported that grain starch has increased from approximately 695 to 717 g/kg while grain protein has fallen from approximately 100 g/kg in 1931 to 87 g/kg in 1991, a decrease of 23%. Since grain makes up approximately one half of total dry weight, the changes in grain starch and protein should also be reflected in whole-plant composition.

In the U.S., corn breeders have traditionally believed that the highest yielding grain varieties were also the most suitable for use as silage, but current literature indicates this attitude should be reexamined. In general, correlations between grain and whole-plant yield are reported to be only marginally significant for modern hybrids. For example, Vattikonda and Hunter[54] conducted a 2-year study for two sets of hybrids (28 and 60 entries) in Ontario, Canada and measured correlations between grain and whole-plant yield of only $r = 0.48$ and 0.50, respectively. The highest grain-yielding hybrid had a 10% lower forage yield than the highest forage-yielding hybrid, and the converse was equally true. Similarly, Allen et al.[55] obtained a correlation of $r = 0.51$ in a trial of similar design and magnitude. Leask and Daynard[56] reported that there were no significant correlations between grain and stover yield among grain hybrids recommended for use in Ontario, Canada. While some studies report highly significant correlations (e.g., Dhillon et al.[57]), the data of Vattikonda and Hunter,[54] Allen et al.,[55] and Leask and Daynard[56]agree with studies by Lorenzoni et al.[58] in Italy, Gallais et al.[59] in France, and Geiger et al.[60] and Utz et al.[61] in Germany.

In hybrid trials conducted at the University of Wisconsin, most correlations among yield and whole-plant quality characteristics have been nonsignificant when hybrids are evaluated over several environments and appropriately grouped into separate trials by relative maturity.[62] The

only significant correlation was between whole-plant yield and whole-plant NDF (r = 0.57) for late-maturing hybrids, but the corresponding correlation was not significant in the early trial. Allen et al.[55] harvested corn hybrids individually at approximately at 1/2 milk line (ML) stage of maturity, and they reported that whole plant yield was positively related to grain yield (r = 0.51) and whole plant NDF content (r = 0.48), and negatively related to percent grain (r = –0.48), IVTD (r = –0.46), and whole plant crude protein content (r = –0.42). Other studies comparing hybrids harvested at a broader range of maturities may detect a larger number of significant correlations, but such correlations may be primarily due to differences in physiological stage rather than true genetic associations between yield potential and quality.[60,63,64] For instance, later-maturing hybrids tend to have lower grain-to-stover ratios, which may lead to higher total fiber content. While there is a tendency for higher grain-yielding hybrids to also have higher total forage yield, whole-plant nutritive value can vary within any given level of grain or whole-plant yield

Whole-plant yield and stover composition are also independent to one another to a large extent.[54,62,65–68] In the Coors[62] study, the only significant correlations involving stover composition were with maturity-related traits and ear percentage, and not yield *per se*. Even though whole-plant composition was not strongly related to stage of maturity at harvest, as hybrids matured, stover NDF increased while stover IVTD, IVNDFD, and protein decreased. Within the narrow range of relative maturities in these trials, grain development apparently diluted the effect of increasing stover NDF and decreasing stover digestibility and protein.

B. Influence of Cultural Practices and Climate on Forage Production

There have been numerous studies on the influence of temperature, light intensity, plant density, planting date, row spacing, and N management on forage quality. Cultural aspects of corn silage production have been summarized by Roth et al.[69] All of these factors can affect the partitioning of dry matter to leaf, stalk, and ear. Several overall conclusions are evident and are summarized in Table 13.4 as modified from Struik[70] and Deinum and Struik.[45] In the Netherlands, extensive investigation of temperature effects at different growth stages have shown that higher temperatures before tassel emergence, if not accompanied by moisture stress, increase total dry matter production, but later in development, higher temperatures have greater influence on rate of dry matter production.[71] Higher temperatures tend to reduce digestibility because of increased cell wall content and decreased CWD of stover tissue. Greater light intensity has the same effect on dry matter production, particularly the grain fraction, but tends to also promote nutritive value of stover tissue by reducing concentration of CWC.[72,73]

Higher planting densities, relative to those customary for grain production, increase whole-plant yields.[59,74–86] Olson and Sander[87] indicated that optimum plant density may differ between corn grain and forage production with higher plant densities favoring forage rather than grain yield. The increase in lodging as a result of increased plant density is less important for silage

TABLE 13.4
Effects of Climatic and Cultural Practices on Yield and Quality of Silage Corn

Factor	Whole-plant Dry Matter Yield	Dry Matter Digestibility	Cell Wall Content	Cell Wall Digestibility
High temperature	+	–	+	–
High light intensity	+	+	–	±
High stand density (including narrower rows)	+	–	+	±
Delayed planting	–	–	+	±
Delayed harvest	–	–	+	–
Increased N rate	+	–	+	±

than for grain production, since maximum dry matter rather than grain production is the greatest concern. Likewise, grain moisture is not as important in silage production as it is in grain production and, therefore, later hybrids with more and larger leaves might be utilized. For example, in Ontario, Canada, it is recommended that planting densities for forage production should be approximately 20% higher than for grain.[88] In New York, Graybill et al.[75] found that total dry matter production increased as the planting density increased from 50,000 to 80,000 plants/ha, the latter being the highest planting density in their study. Optimum plant densities are highly dependent upon environment.[84] Maximum forage yields of modern hybrids have been reported between the range of 79,000[75] and 143,000 plants/ha.[83] Modern corn hybrids tolerate higher plant density stress better than older hybrids in part because of decreased lodging[49] and decreased bareness.[89] Optimum silage yield densities do not vary much among hybrids with fixed, flex, or semi-prolific ear types.[84]

Even though corn forage yield may have a greater optimum plant density than corn for grain, forage quality losses at higher plant density have been reported.[90] Higher densities are usually associated with decreased dry matter content, possibly related to decreased ear percentage and delayed maturity, which may adversely affect preservation during ensiling and lead to decreases in digestibility and intake. As plant density increases, *in vitro* true digestibility decreases.[83,91,92] Sanderson et al.[91] reported that ADF, ADL, and cellulose concentration in whole plant, stalks, and leaf blades increased by 20 to 40% when plant density was increased. Cusicanqui and Lauer[92] found that crude protein decreased while NDF and ADF increased with greater plant density (Table 13.5). Due to the trade-off between yield and quality, silage plant density recommendations in Wisconsin are similar to grain plant density recommendations. The negative relationship between plant density and corn forage quality makes it difficult to recommend a plant density for optimum animal performance based on yield. Others, though, have reported no significant reduction in quality, although trends are often apparent.[74,75,77] Reductions in some measures of nutritive value have been recorded in other studies.[76,77,93–96] The seeming disparity in findings depends on several factors. If nutritive value is measured prior to ensiling, there may be little effect of decreased dry matter or altered grain-to-stover ratios because overall concentration of available carbohydrate may remain relatively constant over densities. However, high moisture and low-starch concentration tend to reduce efficiency of fermentation during ensiling and increase potential seepage losses.[96] Such effects would become more evident in subsequent assessments, particularly as measured in feeding trails evaluating *in vivo* digestibility and dry matter intake. In Canada, planting densities in excess of those that produce a forage with less than 300 to 350 g/kg dry matter face some risk of lower dry matter intakes.[97] Consumption of corn silage by cattle has been shown to decrease significantly as dry matter contents are reduced from 300 to 200 g/kg.[98]

TABLE 13.5
Corn Hybrid Whole-Plant Yield and Quality Response to Plant Density over Six Locations in Wisconsin (1994–1996)

Target Plant Density (plants/ha)	Harvested Plant Density (plant/ha)	Dry Matter Yield (Mg/ha)	IVTD (g/kg)	CP (g/kg)	NDF (g/kg)	ADF (g/kg)	CWD (g/kg)
44500	46200	15.0	780	75	452	220	515
59500	60400	16.5	771	71	465	228	509
74500	74700	17.7	763	71	467	238	472
89500	87100	18.0	765	68	474	237	505
104500	100500	18.3	761	67	481	242	503
LSD (0.05)	1230	0.4	9	4	9	9	NS

Source: Data from Cusicanqui and Lauer.[92]

The effect of delaying planting is very similar to that seen with grain in terms of whole-plant dry matter yield, but there is also a quality penalty. Production practices should emphasize the rapid production of the grain fraction for the same reasons noted previously. Higher dry matter at harvest and greater grain content will promote more effective preservation. Delayed planting may also reduce dry matter digestibility due to an increase in CWC. For example, in British Columbia, Canada, whole-plant *in vitro* digestibility was reduced progressively from 660 to 630 g/kg with delayed planting, and between early May and mid-June, each 1 day delay in planting reduced whole-plant digestible dry matter yield by 1%.[99] Delaying planting date does not affect nutrient uptake of N, P, K, Ca, or Mg corn silage.[100] Delaying planting in northern regions also increases risk of frost damage prior to harvest. Dry matter yields usually decrease after frost due to leaf loss and increased lodging potential. Freezing leads to leaching of cell contents because of cell rupture, thereby reducing soluble carbohydrate and nitrogen.[101] Frost occurrence has shown to increase silage fiber content, reduce dry matter intake, and reduce *in vivo* digestibility.[102]

With modern hybrids, the advantage to narrower rows for silage production lies primarily in a yield increase, as quality is not affected. Studies conducted in the 1960s often did not observe yield advantages with narrower rows,[80,81] although Cummins and Dobson[82] did observe greater yield with narrower rows in one environment. In New York, narrowing row spacing from 76 to 38 cm increased silage yields 4.2%.[85] Under droughty soil conditions no advantage was seen with narrower row spacing for silage yield.[85] Row spacing did not affect IVTD, NDF, CWD or crude protein.[85] Plant density × row spacing interactions for yield and quality did not exist in New York.[85]

Nitrogen recommendations for corn grain production show that optimum N rates were not related to yields, but were a soil-specific characteristic that varied little with year-to-year fluctuations in yield.[103–106] While higher N rates do not seem to significantly affect the yield and nutritional value of corn grain, the quality of corn grown for silage can be markedly affected. Corn forage dry matter yield and crude protein increases with increasing N rate.[83,91,100,107,108] N rate effects on fiber levels and *in vitro* digestibility are inconsistent.[108] Generally fiber levels increase and *in vitro* digestibility decreases as N rate increases, although not as much as yield and crude protein.[83,91] Silage yield response to N rate is measured up to between 122 and 179 kg N/ha.[83,109,110] Special care must be taken with corn silage produced under extremely high N rates. The acute and chronic toxic effects of nitrate accumulated by plants and consumed by animals in silage, as well as "silo gas" produced from plants high in nitrate can be lethal and is well documented.[69]

C. Relationships between Plant Morphology and Nutritional Value

Few quality attributes are consistently associated with morphological characteristics, but much research on the relationship among agronomic and quality traits is suspect due to myriad confounding effects of climate and plant maturity. For example, plant color, height, and leafiness were found to have little relationship to nutritive value in the study of Deinum and Bakker.[65] Somewhat contradictory conclusions were reached by Schmid et al.,[111] who, using 23 corn silages, found significant negative correlations of digestible dry matter, dry matter intake, and average daily gain (when fed to sheep) with percentage of leaves and stalks. Corresponding correlations of quality attributes with percentage of ears were positive. The silages in the latter study, however, were harvested without regard for physiological stage. Dry matter contents ranged from 228 to 558 g/kg, with ear percentage ranging from 11 to 67%. Some care must be taken when reviewing the relevance of such research to the question of whether there are true morphological or physiological associations between agronomic and quality characteristics.

Of the compositional traits influencing nutritive value, lignin and fiber concentrations are most often associated with agronomic characteristics. Larroque and Planchon[112] reported a high correlation of leaf angle with lignin concentration. In their study of eight corn inbreds and nine derived hybrids, the more upright leaves had the highest lignin concentrations. Since modern hybrids have more upright leaves than older hybrids, (thus allowing greater light penetration into the canopy at

higher plant densities[53]), it seems likely that leaf or leaf sheath lignin concentrations may have also increased in modern hybrids.

Many corn breeders also believe that the steady improvement in lodging resistance over the last several decades is due to increased stalk lignification, and several studies have shown that increased stalk lignin is associated with greater stalk strength.[113–118] Stover digestibility decreases with increased lignification, and there is some concern that lodging resistance must necessarily come at the expense of nutritional quality. Albrecht et al.,[119] however, found that selection for increased stalk lodging resistance actually increased nutritive value. Three cycles of recurrent selection in the synthetic variety BS1 were used to produce two improved synthetics, one for increased stalk mechanical strength, and the other for resistance to *Diplodia* stalk rot. Total CWC of stalk internodes was reduced from 616 g/kg for the base population to 520 and 542 g/kg, for the two respective improved synthetics. Corresponding decreases for lignin were from 57 to 42 and 47 g/kg, and *in vitro* dry matter digestibility (IVDMD) increased from 514 to 621 and 594 g/kg. Both selection schemes seemed to increase total nonstructural carbohydrate concentration, which diluted fiber concentration and apparently increased digestibility. Lourenço et al.[120] reevaluated a subset of these BS1 synthetics, as well as several hybrids, planted at three densities and reached similar conclusions. Argillier et al.[63] evaluated 11 flint and dent inbreds crossed to 4 tester lines, and they also showed that root and stalk lodging resistance need not necessarily be at the expense of stover quality. They echoed the contention of Albrecht et al.[119] that stalk lodging resistance was more related to plant health than fiber composition. Healthier plants typically had higher sugar concentration in the stalk, possibly increasing turgor pressure and thereby increasing stalk strength. Wolf et al.,[67,121] evaluated a diverse array of 24 S_2 lines developed for extreme (high and low) levels of stover fiber and lignin, and they found that root and stalk lodging were not consistently associated with stover concentrations of NDF, ADF, or lignin. Melchinger et al.[122] also did not find any significant phenotypic correlations for root stalk lodging with stover digestibility in a study of 22 flint and dent inbred lines and 66 F_1 forage hybrids. From these studies it seems that distribution of cell wall structural components may be equally, if not more important, than overall concentration.

D. Relationships between Pest Resistance and Nutritional Value

Cell wall composition, and particularly lignin, can influence insect and disease resistance in plants.[123–131] In corn, increased concentrations of plant fiber, lignin, and silica are positively correlated with resistance to second-generation European corn borer (ECB) [*Ostrinia nubilalis* (Hübner)] and other insects.[132–140] Corn breeders have effectively selected for resistance to European corn borer for many years,[53] and this may have led to higher concentrations of cell wall carbohydrates and lignin in leaves, leaf sheaths, and stalks of corn. It is not known whether the intense selection for ECB resistance by the hybrid corn industry, prior to the development of transgenic Bt corn, actually reduced nutritive value of modern hybrids, but it seems possible.

E. Corn Germplasm

Corn germplasm is genetically variable for a number of characteristics that influence its suitability as a silage. Maturity, grain-to-stover ratio, grain composition, and stover composition will be reviewed below to illustrate many physiological and genetic aspects of silage productivity and quality.

1. Maturity

Later-maturing genotypes typically have greater dry matter yield associated with increased leaf area indices and leaf area durations.[141] Many agronomists suggest use of slightly later-maturing hybrids for silage than for grain, as long as the crop can be harvested at a suitable whole-plant

moisture for optimum preservation. In cool regions, where daily respiration may be in excess of photosynthesis late in the season, carbohydrates are translocated from vegetative structures to the developing grain, and there is no overall increase in total plant dry matter. Maximum forage yields may be obtained before maximum grain yields.[97]

Maximum whole-plant dry matter yields are typically achieved by the time the milkline is 3/4 down from the top of the kernel.[142–146] If hybrids are harvested for grain at this stage, they may have reduced grain quality and require excessive drying, but as silages they may allow optimal total dry matter production with little sacrifice of nutritive value.[75] Dry matter content of corn forage increases steadily after flowering, and this is due primarily to grain development of the ear. This is associated with a small reduction in fiber and protein concentration due to the dilution effect of the relatively low-fiber, low-protein grain. The change from vegetative to reproductive growth in corn is also accompanied by a decrease in soluble sugar content in the stover (primarily sucrose, fructose, and glucose) and an increase in starch in the grain. Hunt et al.[143] evaluated six genetically diverse corn hybrids for the effects of maturity on ear, stover, and whole-plant composition, and ruminal *in situ* dry matter disappearance (ISDMD, Table 13.6). A progressive increase in whole-plant starch concentration reflected the contribution from the developing ear and depletion of total sugars in the stover. Total available carbohydrate (sugars + starch) also increased from 320 to 376 g/kg, but these results may depend on environmental conditions. In northern climates where grain development occurs under decreasing temperatures, total nonstructural carbohydrate concentrations may not always change appreciably if the existing canopy structure cannot support requirements of developing ears.[10] In such conditions, later varieties, even with less developed ears, may still prove nutritionally equal to earlier-maturing hybrids because the smaller ear sink would draw less on available carbohydrate reserves of stover tissues.[12,79] However, a silage with a high ear-to-stover ratio is still desirable from the standpoint that it allows harvest at a dry matter content sufficiently high to prevent excessive loss of nutrients during ensiling.[96]

Total structural carbohydrate concentration of stover and extent of lignification of fiber components increase with advancing maturity. Nonetheless, on a whole-plant basis concentrations of NDF, ADF, and lignin usually decrease with age as the ear component increases until a dry matter of at least 350 g/kg is achieved. Wiersma et al.[144] evaluated four early-maturing hybrids at five stages of kernel maturity ranging from soft dough stage (240 g/kg whole-plant dry matter) to no visible milkline (420 g/kg whole-plant dry matter). Over this 30-day period, stover NDF and ADF concentrations increased by approximately 320 and 290 g/kg, respectively, while whole-plant NDF and ADF concentrations were reduced by 750 and 440 g/kg by the 1/2 milkline stage (approximately 350 g/kg whole-plant dry matter) and remained relatively constant thereafter. The highest level of whole-plant digestibility occurred during the period from early dent to 3/4 milkline. Similar results are provided by Ganoe and Roth,[145] although they observed that whole-plant fiber content continued to decrease until black layer in one of the two years of their study. The data from Hunt et al.[143] agree with the findings that increases in stover fiber and lignin are compensated for by developing grain to the extent that whole-plant NDF and lignin concentrations are at a minimum near the 2/3 milkline stage. Hunt et al.,[143] however, continued to see improvements in ISDMD as the plants matured, perhaps reflecting their use of a 24-h fermentation rather than the 48-h fermentation used by Wiersma et al.[144] The studies of Wiersma et al.[144] and Hunt et al.[143] are consistent with many others[94,147–150] that on the basis of both yield and quality, corn should be harvested for silage when the milkline is from 1/4 to 3/4 the distance from the top of the kernel. However, these are only rough guidelines, because considerable variation exists among modern hybrids for plant dry matter content at any given milkline stage.[151]

The maturity effect on kernel texture and starch digestibility has only recently been recognized, perhaps because most laboratory assays of quality require thorough pregrinding of sampled tissues. However, *in vivo* digestion studies may better reflect effects of changing kernel texture as corn plant matures, particularly as the kernel reaches physiological maturity (black layer) at approximately 350 to 400 g/kg whole-plant dry matter. Several recent reports indicate that whole-plant

TABLE 13.6
Chemical Composition, *In Situ* Dry Matter Disappearance (ISDMD), and Estimated Total Digestible Nutrients (TDN), of Ear, Stover, and Whole-Plant Samples of Corn Harvested at Three Maturities

Item	Maturity[a]			SEM[b]
	1/3	2/3	1	
Ear:				
Dry matter	496	582	648	4
NDF	264	234	230	4
Lignin	13	11	12	1
Total sugar[c]	67	55	45	1
Starch	508	581	624	6
TDN	802	814	817	2
Stover:				
Dry matter	259	290	331	5
NDF	560	608	642	5
Lignin	35	41	46	1
Total sugar[c]	120	88	90	4
Starch	78	60	42	4
TDN	554	504	468	7
ISDMD	512	471	459	7
Whole plant:				
Dry matter	317	391	454	5
NDF	463	438	445	6
Lignin	30	28	30	1
Total sugar[c]	98	71	66	3
Starch	222	284	310	6
TDN	662	684	682	4
ISDMD	603	588	564	1

Note: Values represent means over six maize hybrids (g/kg).

[a] Maturity rating represents placement of milkline relative to top of the kernel. Rating 1 represents black layer formation.
[b] Standard error of the mean (n = 48).
[c] Fructose, glucose, and sucrose.

Source: Adapted from Hunt et al.[143]

digestibility declines with maturity for corn silage harvested greater than 300 to 350g/kg dry matter.[93,98,152] Delaying harvest to the black layer stage may result in increased passage of starch (in the form of intact kernels) to the duodenum and feces, increased ruminal pH, and decreased flow of nitrogen to the duodenum.[152] Bal et al.[98] reported that digestibility of diets containing 335 g/kg corn silage by lactating dairy cows was lower for corn silage harvested at 420 g/kg dry matter (black layer) than corn silage harvested earlier at whole-plant dry matters of 301 g/kg (early dent), 324 g/kg (1/4 milkline), or 351g/kg (2/3 milkline). In particular, digestibility of starch decreased by 10%, from 941 g/kg to 877 g/kg from early dent to black layer.

As a plant matures, the increase in dry matter content due to starch deposition in the grain also affects extent of protein degradation that occurs during ensiling.[10] Wilkinson[11] found that at higher dry matter concentrations, degradation of protein to nonprotein nitrogen during ensiling was reduced and suggested that, since non-protein nitrogen is completely degradable in the rumen, harvesting

corn at higher dry matter contents may allow an increased supply of undegraded, bypass plant protein to the abomasum.

2. Grain-to-Stover Ratio

There is considerable genotypic variation for plant morphology. Each plant part has distinct quality characteristics (Table 13.7), and altering plant architecture is one possible method of improving nutritive value. Since grain is the primary contributor to the total digestible nutrients (TDN), many maintain that hybrids combining high total dry matter yield with high ear percentage are the optimum silage hybrids. While this belief seems entirely logical, and may still provide the simplest operational guideline for selecting silage hybrids, several studies show that the relationships between grain-to-stover ratios, total dry matter yield, and nutritional value are not as predictable as first thought.

Grain-to-stover ratios can vary from essentially 0 (using sterile or barren germplasm) to above 60%. Even if grain formation is prevented, total available (nonstructural) carbohydrate in stover remains relatively high, and some have suggested that sterile corn may even be a productive and high-quality silage in certain circumstances.[153] Barren plants accumulate more soluble carbohydrate in stalks than normal plants, and total dry matter and digestibility of the stover of barren corn may even exceed that of normal corn.[153–158] However, total nonstructural carbohydrates and whole-plant digestibility are reported to be greater in fertile than barren plants.[153,155,158] In fertile plants, nonstructural carbohydrates are mostly in the form of starch in the grain, while for barren plants they take the form of soluble carbohydrates in the stalk and leaf tissue.[153,155,156] Several feeding trials have examined the *in vivo* digestibilities of sterile hybrids, and the results reflect the apparent energy tradeoff between soluble carbohydrates and starch. For example, in a lactation trial spanning three cropping seasons, Burgess and Nicholson[159] could detect no difference between fertile and barren silages in dry matter intake, dry matter digestibility, or milk yields. In a similar comparison involving sheep, no differences in dry matter digestibility were detected.[153]

Prevention of complete grain development limits whole-plant production potential in most instances. In climates with higher temperatures and light intensities, grain filling is primarily due to current photosynthesis.[165] Dry matter yield in the U.S. Corn Belt has been shown to be limited by

TABLE 13.7
Distribution of Dry Matter and Quality of Various Plant Parts of the Silage-Corn Hybrid Circé Grown in the Netherlands

Tissue	Contribution to Plant Yield (%)	Digestibility of Organic Matter[b] (g/kg)	Cell Wall Digestibility[c] (g/kg)	Cell Wall Content[d] (g/kg)
Tassel	1.3	476	531	778
Cob	10.1	684	707	648
Husk + shank	11.8	655	726	765
Mid-rib	2.1	563	622	768
Leaf mesophyll	9.2	767	828	644
Leaf sheath	4.5	556	632	805
Rind of stem	13.2	544	557	693
Pith of stem	4.3	736	733	514
Kernel	43.5	886	937	99
Whole plant	100.0	754	707	434

[a] Apparent digestibility.
[b] True digestibility.
[c] Based on organic matter.

Source: Adapted from Deinum and Struik.[45]

sink capacity,[157,158] and the photosynthetic rate of barren plants may reach only 55% that of normal plants one month after flowering.[160] Barrenness reduces total whole-plant dry matter, and the reduction can be as much as 27% even though the dry matter yield of stalks and leaves may increase by as much as 59%.[154–158,161] Somewhat different growth patterns are observed in areas of the northern U.S., northern Europe, and Canada with lower temperatures and light intensities, where rate of grain filling may exceed current photosynthetic capacity of stover.[155] In Great Britain, Bunting[162] compared dry matter production and distribution of fertile and sterile plants and estimated that one third or more of final grain weight was derived from photosynthates produced within 1 month after flowering and temporarily stored in stover. Grain filling may not be as essential for maximizing total dry matter yields in such areas of lower solar irradiance and cooler autumn temperatures. As a result, yield comparisons of barren and fertile plants performed in Great Britain and similar climates are often less dramatic than those in the U.S. Corn Belt.[156,159,162,163] Variable conclusions relating extent of grain fill to whole-plant productivity and quality are possible, and disparities depend primarily upon where and under what conditions such studies were conducted. On the basis of dry-matter yield, however, barren hybrids have shown little real promise.[164] Even though there may be a tradeoff between stover quality and ear development, at least in the U.S. Corn Belt, an effective sink is still required to maximize both total dry matter accumulation and whole-plant quality because kernels have higher protein, lower concentrations of cell wall carbohydrates, and are more digestible.[161]

Comparisons of barren with normal corn do provide a means for relating genotypic variation in grain-to-stover ratio to the physiological basis of nutritive value. Grain develops at the expense of stover nutritive value, and negative correlations between stover digestibility with ear content or positive correlations between stover fiber and ear content are common in the literature.[59,60,62,65,68,74,155,156,162,165–169] If the genotypes under study have a range in maturity, though, some caution is needed in interpreting correlations because it is nearly impossible to separate out the effects of maturity on such source-sink relationships.

Most evidence to date suggests that grain percentage, while important, does not play the sole role in determining whole-plant forage quality. While increasing grain yield may be the most effective way of increasing total dry matter yield, the nutritive value of whole-plant corn, at least in northern temperate environments, seems to be only partially dependent on the grain component as long as dry matter content permits effective fermentation in the silo.

3. Brown-Midrib Silage

Several low lignin genetic mutants have been discovered in corn, and their use in silage studies demonstrates potential for genetically modifying fiber composition to improve nutritive value of silage. The first brown-midrib plant was discovered at the University of Minnesota in 1924.[170] Subsequently, this gene was named *bm1*. Since then three additional brown-midrib mutants have been discovered: *bm2*,[171] *bm3*,[172] and *bm4*.[173] All four brown-midrib genes originated from natural populations and segregate independently in a simple recessive Mendelian fashion.[174]

The lower lignin content of brown-midrib genotypes is due to lower concentrations of several hydroxycinnamates, and the specific effect depends upon the particular enzymes associated with each brown-midrib allele.[175] The *bm1* allele causes an alteration of the enzyme cinnamyl alcohol dehydrogenase (CAD), which converts cinnamyl aldehydes to the corresponding alcohol form that is incorporated into the lignin polymer.[176] The *bm3* allele causes a truncation or deletion in the gene encoding caffeic acid-O-methyl transferase (COMT),[177] which leads to an increased 5-hydroxyguaiacyl concentration in the lignin polymer. Tagging and cloning of the *bm2* gene is underway, and the associated enzyme(s) will likely be identified in the near future.[178] At this time it is known that the *bm2* genotypes have a higher syringyl content and a lower guaiacyl content than normal corn, and they do not produce 5-hydroxyguaiacyl components.[179] Less in known about the *bm4* allele, but it is reported to have a similar *p*-coumaric/ferulic acid ratio as normal corn but a reduced syringaldehyde content.[180]

There have been several recent reviews describing the extensive research involving brown-midrib forages,[181,182] and the following discussion represents only a brief summary of the efforts by nutritionists and breeders to evaluate the potential of brown-midrib varieties. The *bm3* allele, in particular, has been exhaustively studied by plant and animal scientists because it typically reduces lignin and increases digestibility to a greater extent than other such mutants.[116,117] Stems, leaf blades, and leaf sheaths are all affected.[117,183–185] Lignin concentration of *bm3* genotypes is reduced on a cell wall (NDF) basis as well as a on a total dry matter basis.[183,185,186] Both NDF and ADF concentrations are usually lower for *bm3* mutants,[27,116,117,183,185–188] although overall fiber reductions are harder to detect in cooler, northern climates, and such effects depend to some extent on genetic background.[184]

In vitro digestibility of *bm3* genotypes is greater than normal counterparts, but the effect is primarily encountered in the stover fraction.[27,116,117,183,186,187] Whole-plant digestibility may not be increased in *bm3* germplasm because of reduced grain yield. The increase in *in vitro* stover digestibility is associated with an increase in digestibility of NDF, ADF, and cellulose fractions.[188–192] In feeding trials, *bm3* genotypes showed greater *in vivo* digestibilities in studies of sheep, goats, beef cattle, and milk cows, where there were general increases in feed intake and liveweight gain, and less consistent increases in feed efficiency (weight gain relative to feed intake).[188–199] Effects on milk production in dairy cows seem more difficult to detect. In some cases, feeding *bm3* silages significantly increased milk production,[193,194,196,200–202] but in other studies, *bm3* silages had no effect or only a slight trend toward higher milk yield.[195,197,198,203] Allen et al.[199] concluded that even though higher milk yields have not been consistently obtained with *bm3* silages, most studies have shown increases in either milk yield or body weight gain, suggesting that feeding response to *bm3* silage is a function of rumen fill limitations to feed intake. The more rapid degradation of *bm3* silages in the rumen allows greater intake, and the energy derived from the fermentation of cell wall carbohydrates can then fill the most immediate energy demands; i.e., those of milk production or body condition. Physical fill in the rumen is more likely to limit intake when cows are in negative energy balance; e.g., early in lactation when cows are mobilizing body reserves to meet energy requirements. Experiments involving cows with high dietary energy requirements, either cows early in lactation or high-producing cows, typically show increased dry matter intake of *bm3* silages, and the intake effect increases as the proportion of corn silage in the ration increases. Furthermore, the relative advantage of *bm3* silages in terms of intake and milk yield is linearly related to milk yield of the cows under study.[202]

Ration-balancing strategies need to be reevaluated when using *bm3* silages to account for their higher fiber digestibility. Merely replacing conventional corn silages with *bm3* silages on an equivalent dry matter basis may well have adverse consequences, such as depressed milk fat tests. One possible strategy was evaluated by Bal et al.[204] Rather than keeping the forage portion of the diet constant for the silages they compared, they increased the dietary forage to 600 g/kg for the *bm3* silage versus 470 g/kg for the normal silage. (The forage portion was 2/3 *bm3* or normal corn silage and 1/3 alfalfa silage). Intake was not affected, even though the *bm3* diet had 28% more forage in the diet, suggesting that it may be possible to substitute *bm3* forage for other more expensive sources of dietary energy. However, the *bm3* diet also reduced milk yield by 1.4 kg/day, suggesting that the forage or concentrate portion of the ration needed further refinement.

Agronomic evaluations of *bm3* hybrids have been discouraging because of relatively low growth rates, poor early-season vigor, increased lodging, delayed flowering, and poor grain and forage yields.[115,193,205–209] For example, Gentinetta et al.[206] evaluated 21 near-isogenic normal and *bm3* hybrids, where average lignin concentrations were 60 g/kg and 38 g/kg, respectively. Corresponding stalk lodging measures were 3.8% and 12.5%, and the forage yield of normal hybrids was 17.9 Mg/ha compared with 14.4 Mg/ha for *bm3* hybrids. In a similar study of 14 near-isogenic counterparts, Allen et al.[192] reported that the average forage yield of *bm3* hybrids was 13% lower than their normal counterparts, 16.0 vs. 18.3 Mg/ha. Slower growth rates and later flowering dates may delay harvest by a week or more when compared with normal corn, and, on these criteria alone, *bm3* hybrids are unacceptable in many silage growing areas. The proportion of the whole plant

provided by the ear is usually less for *bm3* than normal genotypes.[193,208] Stalk crushing strengths are appreciably lower for *bm3* genotypes, and this is associated more with reduced stem weight than any reduction in rind thickness.[208,210]

Attempts to use the *bm3* allele alone by backcross conversions have been frustrating, and some breeders have attempted to create *bm3* pools incorporating modifiers from well-adapted germplasm.[115,205] Such attempts have not yet met with much success. For example, Miller et al.[115] evaluated 130 normal and 130 *bm3* S_1 lines from three populations segregating for the *bm3* allele. On average, the *bm3* genotypes averaged 77% of grain yield and 84% of whole-plant yield of normal genotypes. No *bm3* genotypes produced as much grain or whole-plant yield as the best normal genotypes. The authors suggest that selection in normal populations for quality attributes offers more potential than selection for better yield characteristics in *bm3* backgrounds. Using this approach, Falkner et al.[211] selected for lower lignin concentrations in normal germplasm and produced inbred lines with lignin concentrations and digestibilities equal to *bm3* genotypes.

4. High-Quality Protein Corn

The *opaque2* (*o2*) and *floury2* (*fl2*) alleles elevate levels of lysine and tryptophan in corn grain,[212] and their discovery prompted animal scientists to investigate their potential use in feeds, primarily for monogastric animals, but there also has been some interest for their use in corn silages. The *o2* and *fl2* alleles are inherited as simple recessives, although modifiers exist.[172,213,214] The *fl2* allele was evaluated initially by several breeding programs, but its use has been discontinued because the *o2* genotypes offered more potential. Initially the effects of the *o2* allele were deleterious and included lower grain yields due to decreased endosperm size, greater incidence of ear rot, increased grain moisture content, and poorer germination. High-quality protein *o2* corn also has a soft chalky endosperm, which can lead to increased damage by stored grain insects and higher amounts of kernel breakage than the flint or dent types. However, several decades of intensive breeding at CIMMYT and elsewhere have overcome many of these deleterious characteristics.[214]

Lysine is among a group of limiting amino acids, but for increased lysine of *o2* genotypes to be beneficial for ruminants, lysine must escape rumen degradation and be absorbed directly by the small intestine. Unfortunately, there have been few silage evaluations of high-lysine genotypes. In a 40-cow lactation study comparing *o2* and normal genotypes, Andrew et al.[215] detected no effect on milk production or composition, feed intake, or feed efficiency. There were some differences in digestibilities of ADF, organic matter, crude protein, ether extract and nitrogen-free extract, but differences tended to cancel out any overall effect of any one genotype. In general, feeding value of *o2* corn was no different from normal. Beek and Dado[216] reported that milk yield, milk fat and protein contents, and body weight were not affected by feeding high-lysine silage to dairy cows, although dry matter intake was 9% higher than for the normal silage diet. A similar comparison involving beef cattle[217] also showed that there was no metabolic advantage for *o2* genotypes, and it was concluded that *o2* silage did not have as high a nutritional value for finishing steer calves as normal silage.

Another, and perhaps more promising advantage to high-lysine genotypes might be their altered kernel texture, which may improve starch utilization by ruminants.[218] The *o2* allele alters the protein matrix surrounding starch granules in a manner than seems to increase either or both the rate and extent of starch digestibility. Dado and Briggs[219] reported that 6 and 12 hr *in vitro* starch digestibilities of seven high-lysine varieties averaged 170 and 70 g/kg higher than the normal check hybrid. Beek and Dado[220] reported that *in vivo* starch digestibilities of high-lysine silages were approximately 50 g/kg higher than normal corn when fed to lactating holsteins.

5. High-Oil Silage

The oil concentration of kernels from conventional corn hybrids ranges from 35 to 50 g/kg oil, and varieties with greater than 60 g/kg are considered as high-oil types.[221,222] Corn with elevated oil

concentration has a higher energy density because the calorie content of oil is approximately 2.5 times as great as that of carbohydrates. As a result, feeding grain of high-oil corn has been effective in increasing daily gains and feed efficiencies in poultry, swine, and dairy cows,[223] and high-oil corn has attracted some interest as a silage. Before the advent of the TopCross™ high-oil hybrids, a severe yield penalty often accompanied elevated oil levels. For example, 24 cycles of selection for high oil in the Alexho Synthetic was effective in elevating grain oil content from 51 to 170 g/kg, but grain yields declined from 4.8 to 3.2 Mg/ha.[224] On the other hand, Miller et al.[225] increased oil content in a Reid Yellow Dent synthetic from 4.0 to 9.1% by seven cycles of recurrent selection without reducing grain yield.

There is little evidence that high-oil silages have any consistent nutritional advantage over normal corn, and this holds true for both the older high-oil hybrids and the newer, more productive TopCross™ varieties.[226–230] This is not surprising, since the kernel portion of the plant makes up approximately 50% of the total dry matter yield, and oil concentrations in the kernel are relatively low to begin with. For example, doubling the oil concentration in the kernel from 40 g/kg to 80 g/kg only represents a change in energy density for 2% of the total dry matter of a silage hybrid. Considering the yield penalty, it is not likely that high-oil silages have any unique potential.

6. Lfy Corn

There has been considerable interest in using the dominant *Lfy1* allele to make silage hybrids, which are known as Lfy hybrids. The *Lfy1* allele tends to increase the number of leaves above the ear by four or more when compared with normal corn.[231] Extra leaves may possibly increase plant photosynthate production during grain production, thereby increasing both grain and whole-plant yield.[231] Several Lfy hybrids are widely marketed in the U.S. and Canada, and approximately 16% of North American silage production is from Lfy hybrids.[232]

Closer scrutiny of individual Lfy silage hybrids, however, has not been consistently encouraging. Dwyer et al.[233] compared two Lfy hybrids to a commercial check in Ottawa, Ontario, and reported that Lfy genotypes were able to accumulate higher levels of soluble sugars in the upper canopy, possibly increasing palatability and readily available energy. They also suggested that Lfy hybrids might require lower population densities to maximize digestible dry matter yield. In subsequent evaluations of three commercial Lfy and two normal hybrids, Dwyer et al.[232] found little difference in TDN on either a stover or ear basis between Lfy and normal hybrids, regardless of planting density. Total whole-plant yield and digestible plant yield tended to be higher on average for the Lfy hybrids.

In animal feeding trials, Kuehn et al.[234] and Bal et al.[235] were not able to detect any overall advantage to feeding a Lfy versus normal hybrid to lactating dairy cows. In the Bal et al.[235] study, total tract digestibility of organic matter and ADF were lower and starch was higher for the Lfy hybrid. There was a 15% increase in 24-hour ruminal starch degradation for the Lfy hybrid. Presumably, this was caused by the soft kernel texture of the particular Lfy hybrid evaluated and was not related to leafiness *per se*, although more such studies are needed on the effect of kernel texture. At this time it is not clear what advantages, other than a possible increase in whole-plant yield, the *Lfy1* allele will confer to corn hybrids harvested for silage.

7. Waxy Endosperm

The starch in normal corn is typically 75% amylopection (highly branched chains of glucose molecules) and 25% amylose (straight chains of glucose molecules). The waxy phenotype, conditioned by the recessive *wx1* allele, is characterized by the complete replacement of amylose by amylopectin.[213,223] Theoretically, increasing the amount of branching may increase starch digestibility, but experimental verification of such a general effect has been hard to come by even for monogastrics.[236]

Kiesselbach[157] noted that the waxy endosperm is also an ineffective sink relative to starchy endosperm. Changing the endosperm from *wx1* to normal increased grain yield by 3.3%, increased whole-plant yield by 0.7%, and increased grain-to-stover ratio by 6%. Fergason[237] estimated that the grain yield penalty due to the *wx1* allele is approximately 3.5%. While the feeding value of waxy corn grain has been under intensive study for many years, and it is grown on 0.3 to 0.4 million ha annually,[223] there is little information other than testimonials relating quality attributes to normal corn when used as a silage. The only known study in the literature, that of Tourbier and Rohweder,[238] showed no consistent differences in forage quality between near-isogenic waxy and normal hybrids.

8. Sweet Corn and Popcorn

The poor agronomic performance of sweet corn and popcorn, when compared with normal field corn, is a serious disadvantage for silage production. Starchy endosperm of normal corn is a more effective sink relative to mobilization of soluble carbohydrates from stover. Kiesselbach[157] showed that changing endosperm from sugary (allele not identified) to dent produced a 9% increase in stover dry weight, a 14% increase in whole-plant yield, and a 10% reduction in proportion of stover to grain. Kurle et al.[239] noted that whole-plant dry matter yields of both sweet corn and popcorn were approximately 40% lower than dent corn, and that yields of crude protein and digestible dry matter were at least 50% lower than dent corn.

Other sugar mutants exist, such as the *sugary-Brawn2* (*su-Bn2*) allele, that are more intermediate in phenotype, and may, therefore, prove more agronomically acceptable.[240,241] Studies on the utility of *su-Bn2* silages have, however, not been completed. Sugary-type genotypes do have a serious disadvantage because of their characteristically low grain dry-down rates.[240] For sugary genotypes to reach a whole-plant dry matter content of at least 300 to 350 g/kg, they must be harvested at a more advanced physiological stage. Not only does this delay harvest and increase the risk of spoilage,[239,241] it may lead to reduced stover quality as a result of increased fiber and lignin concentrations.

9. Other Silage Types

Profusely-tillering corn varieties have been developed in Canada that have greater total dry matter production than non-tillering varieties, although the advantage was consistent only under lower than normal plant densities.[242–245] The assessment of comparative quality is difficult because isogenic stocks were not used, and the tillering and non-tillering genotypes may have been from divergent backgrounds. In addition, tillering types in past studies had lower grain-to-stover ratios, and whole-plant moisture content at harvest usually differed, even though the two types may be of similar maturity.[242] Moisture differences may well influence silage preservation and thereby affect *in vivo* digestibility. Based on limited trials to date, tillering types show no significant nutritive advantage in composition,[243,246] or in digestibility of dry matter, energy, and crude protein.[243] Yet tillering types, because of their high dry-matter yield potential, may have significantly greater production of digestible nutrients per hectare.[150,242]

There are a number of dwarfing genes for corn,[213] both dominant and recessive, that may influence nutritive value because they potentially increase grain-to-stover ratio. There are few studies, however, documenting any improvements in quality. Ramsey[247] compared dwarf and normal varieties in yield and feeding trials, but found no difference in either whole-plant yield or quality as reflected in intake or digestibility of dry matter and protein. Byers et al.[248] detected no differences between dwarf and normal varieties in dry matter intake, milk production, or body weight changes in a study of 27 lactating dairy cows. The dwarf variety used in this trial (Illi Dwarf 513) did have higher ether extract and lower crude fiber concentrations. Byers et al.[248] further evaluated four dairy steers to determine coefficients of digestibility and TDN, and they found the dwarf variety had

significantly higher TDN than the normal corn silage. The dwarf variety had significantly lower whole-plant yield, however, and on the basis of TDN/ha, the normal corn variety was superior. Dwarf corn will not likely be used as a forage because of the reduction in whole-plant yield and lack of evidence for substantial improvement in nutritive value.[164]

Autotetraploid corn, first developed by L. F. Randolph, has low grain yield due primarily to reduced seed set,[249,250] but if total dry matter yields were found to be significantly greater than diploid corn, it may have some use as a silage. Atlin and Hunter[251] evaluated three autotetraploid and four diploid synthetics in southern Ontario for forage characteristics. There were no consistent differences among the synthetics for total dry matter yield, although proportion of total yield harvested as grain in autotetraploid synthetics was only 65 to 75% that of diploids. There were also no consistent differences among ploidy levels for IVDMD or crude protein. Digestibility was generally greater for autotetraploid stalks, perhaps reflecting a less effective grain sink, but lignin concentrations did not differ among ploidy levels. Autotetraploid genotypes were generally equivalent to diploids in both forage productivity and quality in all but one respect. Autotetraploids had a significantly lower dry matter content at harvest, which was probably due to their lower grain-to-stover ratio.

Teosinte-derived germplasm has received limited attention for potential forage use. There is only one known study of yield and quality attributes of teosinte germplasm. Barrière et al.[252] attempted to improve both total dry matter production and protein concentration in a population formed from a corn × teosinte (*Zea mays* ssp. *mexicana*) cross followed by one backcross to corn. The corn parents were two early flint synthetics. Average protein concentration of the resultant germplasm was more than 130 g/kg, which was greater than that of the check hybrid. Average whole-plant protein concentration of topcrosses involving S_1 progenies from these populations was higher than check hybrids, but the poor grain yield was reflected in relatively low protein yield/ha.

IV. GENETIC VARIATION AND SELECTION

A. Germplasm

Corn germplasm with adequate grain yield potential will probably form the genetic base for any future efforts to enhance forage potential in the U.S. Many U.S. farmers and livestock producers grow corn for both grain and silage. They usually wait until harvest to decide which fields are to be used for each purpose.[253] This flexibility is appreciated, because at planting it is difficult to predict overall forage needs later in the year or know the condition of the corn crop at harvest. Acreage of silage production will increase when perennial forage legume production is reduced due to winter-kill or drought, or when moisture stress or early frost limits corn grain yield. However, when adequate forage from other crops is readily available and corn grain yields are adequate, producers may prefer the option of selling their grain production in a readily available cash market. In the near future, U.S. corn varieties will remain dual purpose, with most emphasis on grain rather than forage production. In northern Europe, however, where most silage breeding is concentrated, the ideal silage hybrid is expected to not only have the full complement of grain yield and defensive traits, but also (1) high forage yield, (2) high intake potential, (3) high digestibility as well as rate of digestion, (4) optimum concentration of minerals, protein, vitamins, and nonstructural carbohydrates, and (5) excellent cold tolerance and early season vigor. It should be expected, then, that sources of germplasm for improve foraged characteristics in U.S. corn will involve those already in current use with some limited introgression of germplasm from northern temperate regions where forage corn quality and productivity has received more attention.

In the few U.S. studies to date, several researchers have noted the significant range in quality attributes among sources of germplasm and demonstrated the ability to genetically modify fiber composition of corn plants. At the University of Wisconsin, two populations, WFISIHI and WFISILO have been developed by two cycles of divergent S_1 family selection to increase the range

in fiber and lignin concentrations of stover tissue.[67,121,134,139,140] The intent of the selection program was initially to evaluate the effect of fiber content and composition of leaf sheaths on insect resistance, but the research also showed how fiber content and composition can be modified. As a result of divergent selection, the two populations differed significantly for NDF, ADF, and lignin concentrations in all stalk tissues (Table 13.8). The difference between WFISILO C2 and WFISIHI C2 exceeded 100 g/kg for NDF and 10g/kg for lignin for stalk and leaf sheath tissue, which were the tissues initially evaluated during selection. Based on S_1 family evaluations during cycle 1, broad-sense heritabilities for all components in both stalk and leaf sheath tissue were high, exceeding 80% in most instances.[140] Wolf et al.[67,121] testcrossed selected S_1 families from WFISIHI and WFISILO, and it was evident that selection among inbred parents for leaf sheath and stalk composition was effective in creating hybrids with altered composition and digestibility when harvested as silage.

Genetic variation for both stover and whole-plant quality has been detected in several other forage trials using both inbred lines and hybrids.[50,53,59,65,67,114,121,141,253–262] As a result, several public research and extension programs in the U.S., such as those in Michigan and Wisconsin, have initiated widespread silage evaluations for both yield and quality as part of their overall hybrid evaluation programs.[46] These studies should be reviewed while noting the region of the test and the agronomic acceptability of the material under evaluation. The difficulty in interpreting ranges of quality attributes is because, in some studies there is a wide range in maturity at harvest as reflected in dry matter content, and in others there is a wide range in overall productivity as measured by forage and grain yields and ear content. Nonetheless, certain trends are evident. Ranges in stover CWC and digestibilities are as great as, if not greater than, those seen in the whole plant, indicating that both stover composition and grain content contribute to nutritive value. In U.S. studies where maturities and yields indicated that commercially adapted hybrids were being evaluated, there was a relatively narrow range in measures of quality. For example, the irrigated trial of Hunt et al.[114] provided one of the largest ranges in whole-plant digestibility for U.S. hybrids, approximately 70 g/kg; however, their digestibilities were derived *in situ,* and may not be directly comparable to others. The Canadian[53,258] studies also show comparatively large ranges in stover and whole-plant digestibility as do those from the Netherlands[65,66,256,257] and France.[50,141]

There are greater ranges in stover cell wall content and digestibility among inbred lines than among hybrids, and this suggests that screening among at least the most elite parental inbreds for nutritive value may provide improved forage hybrids for the northern Corn Belt. At the University of Wisconsin, current public and historically important inbred lines were evaluated for stover composition and digestibility (Tables 13.9 and 13.10).[261] The planting dates were staggered to insure that the inbreds would be at approximately the same physiological stage at harvest. Among the early and late-maturity inbreds, there were significant differences in nutritive value, even when comparing inbreds at approximately the same milkline rating or dry matter content. In the early trial, for example, inbreds W629A and W845 had approximately the same milkline ratings but differed significantly for IVTD (716 and 779 g/kg) and CWD (538 and 611 g/kg). It is also evident that there is variation for composition and digestibility among U.S. inbreds, and germplasm derived from inbreds such Mo17 and H99 might prove useful in silage breeding programs.

In one of the most thorough analyses of inbred composition, Lundvall et al.[263] and Jung et al.[264] evaluated stem internodes and leaves from 45 inbred lines for a number of stover characteristics at both early (mid-silk stage) and late (physiological maturity) harvest dates, followed by an analysis of digestion kinetics of stem internodes that had been harvested at mid-silk (Table 13.11). While there was significant variation among inbreds for 48-h *in vitro* digestibility at both harvest dates, the correlation between early and late harvest dates for stem internode composition was not significant for NDF, and the correlation was significant but quite low for digestibility. Similarly, the correlation between stem internodes and leaves was not significant for NDF and only weakly significant for digestibility. Based on these results, selection for improved quality of inbreds requires total stover sampling near physiological maturity. The composition and digestion kinetics did show

TABLE 13.8
Whorl, Stalk, Leaf-Sheath, and Blade Composition (g/kg) of WFISILO and WFISIHI Corn Populations after Two Cycles of Divergent S1 Recurrent Selection for Fiber and Lignin

	Whorl			Stalk			Leaf Sheath			Leaf Blade		
Entry	NDF[a]	ADF[b]	Lignin d[c]	NDF	ADF	Lignin	NDF	ADF	Lignin	NDF	ADF	Lignin
WFISILO C0	586	301	25	653	396	52	606	328	31	550	280	26
WFISILO C1	585	300	23	602	359	44	589	313	29	543	273	22
WFISILO C2	589	303	23	584	345	43	575	313	28	516	259	21
WFISIHI C0	593	303	25	671	406	57	647	357	37	557	284	25
WFISIHI C1	599	308	26	699	427	62	675	372	39	563	287	24
WFISIHI C2	603	307	25	690	420	59	680	371	39	580	293	27
B73	541	266	20	577	329	38	593	328	33	525	262	23
DE811	550	275	22	613	369	35	647	361	34	558	292	26
LSD(0.05)	8	8	2	17	15	2	16	14	2	15	10	4

[a] NDF = neutral detergent fiber.
[b] ADF = acid detergent fiber.
[c] Lignin = permanganate lignin.

Source: Data from Ostrander et al.[139]

TABLE 13.9
Stover Composition for Early-Maturity Public Inbred Lines

Inbred	Milk-line[c]	H_2O	Stover Composition (g/kg)[b] NDF	ADF	LGN	CP	IVTD	CWD
A554	2.7	694	617	311	40	85	741	579
A641Ht	2.2	641	591	283	39	108	758	587
A654	3.0	725	592	283	38	101	761	596
A679	2.5	718	626	325	42	90	755	610
A683	2.2	709	577	282	40	90	757	577
ND246	2.8	642	620	345	42	102	732	566
F2	2.5	739	570	300	41	98	737	537
195A SU-BN2	3.3	739	578	299	39	101	737	544
CM105	2.7	693	638	322	42	101	734	583
NY821	2.2	727	600	289	37	100	753	588
RD3501 (W182BN type)	0.7	705	583	293	41	92	761	590
RD4502 (NY821 type)	3.0	719	602	291	40	87	753	591
W59E	0.7	607	604	306	40	91	772	622
W83	1.3	685	610	303	36	96	779	638
W117Ht	2.2	758	543	252	33	110	770	572
W153R	3.3	679	537	239	30	123	795	617
W182BN	2.5	759	574	285	39	99	751	566
W629A	2.2	718	614	310	44	81	716	538
W703	0.7	668	598	305	42	92	761	599
W845	2.0	743	568	277	41	73	779	611
MEAN	2.2	703	592	295	39	96	755	586
LSD (0.05)	0.9	46	48	32	4	13	19	48

[a] Averaged over two locations Wisconsin (Madison and Arlington) in 1991.[261]
[b] NDF = neutral detergent fiber; ADF = acid detergent fiber; LGN = permanganate lignin; CP = crude protein; IVTD = *in vitro* true digestibility; CWD = cell wall digestibility.
[c] A milkline rating of 0 indicates black layer and 4 indicates early dent stage. Ratings of 1, 2, and 3 indicate the position of the milkline as 1/4, 1/2, and 3/4 from the base of the kernel.

that, at least at mid-silk stage, several nonmutant inbreds (e.g., B77 and R227) have NDF concentration and rate and extent of NDF digestion values equal to or superior to brown-midrib inbreds. Jung et al.[264] also recommended that single short (12 to 36 h) and long (96 h) fermentation times be used to identify lines with both rapid rates and high extents of fiber digestion.

B. Combining Ability, Heritability, and Selection

In one of the first studies of combining ability for silage characteristics, Roth et al.,[254] using a diallel among eight corn inbreds, found that general combining ability (GCA) for IVDMD, protein, ADF, ADL, and CWC were larger than those for specific combining ability (SCA). More recently, in a series of studies in Germany starting with Dhillon et al.,[57] GCAs and SCAs were estimated for silage and grain characteristics. Inbreds were divided into two sets, six flint lines and six dent lines, corresponding to the heterotic groups that provide the most effective grain and forage hybrids in northern Europe.[265,266] These inbreds were crossed in diallel fashion to produce 66 hybrids that were analyzed for yield and maturity characteristics. The researchers took the precaution of harvesting all hybrids in the forage trials at a constant number of days from mid-silk to minimize any differences in physiological stage. Both GCA and SCA contributed significantly to genotypic variation for nearly all agronomic traits measured, including stover yield, whole-plant dry matter yield, and harvest

TABLE 13.10
Stover Composition for Medium to Late-Maturity Public Inbred Lines

			Stover composition (g/kg)[b]					
Inbred	Milk-line[c]	H_2O	NDF	ADF	LGN	CP	IVTD	CWD
A619	1.5	686	620	315	34	94	735	573
A632Ht	1.2	635	644	326	33	81	737	591
A635	0.7	504	663	333	37	92	728	591
B37	1.8	716	608	303	34	95	733	562
B73	2.0	638	600	301	33	110	751	585
MO17Ht	2.7	684	553	275	25	93	767	579
W64A	2.2	684	630	319	35	91	728	570
W540	2.8	691	580	288	34	118	727	531
W552	1.5	666	630	318	37	89	722	559
W570	2.0	622	610	307	33	96	734	565
WX6007 (BS13)	2.2	666	634	320	30	70	740	590
OH43	2.7	728	556	272	29	124	749	549
H99	1.7	555	574	294	21	89	781	619
DE811	1.2	690	628	313	35	101	731	573
SDP312	1.0	702	616	315	32	90	738	575
RD5506 (A619 type)	2.3	661	524	264	26	113	772	566
RD5507 (A619 type)	2.3	693	598	298	32	100	740	567
RD5508 (A619 type)	1.8	693	618	310	31	106	743	585
B90	2.7	696	604	300	31	94	741	572
MEAN	1.9	664	605	304	32	97	742	574
LSD (0.05)	1.0	52	38	19	3	14	14	16

[a] Averaged over two locations Wisconsin (Madison and Arlington) in 1991.[261]

[b] NDF = neutral detergent fiber; ADF = acid detergent fiber; LGN = permanganate lignin; CP = crude protein; IVTD = *in vitro* true digestibility; CWD = cell wall digestibility.

[c] A milkline rating of 0 indicates black layer and 4 indicates early dent stage. Ratings of 1, 2, and 3 indicate the position of the milkline as 1/4, 1/2, and 3/4 from the base of the kernel.

index. SCA, but not GCA, was significant for ear yield. Genotype by environment (G × E), GCA × E, and SCA × E, were significant for these traits as well, but the latter interaction was only observed in the forage trials for stover dry matter yield and harvest index. Variance components for SCA were larger than those for GCA for ear, stover, and whole-plant dry matter yields indicating the likely influence of dominance effects on both grain and whole-plant heterosis. Narrow-sense heritabilities for stover, whole-plant yield, and harvest index were higher than the heritability for grain yield (h_n^2 = 55.8, 28.9, 55.9, and 11.6%, respectively). In a companion report[165] examining the same field materials at silage harvest, quality evaluations were provided for stover samples, including estimates of NDF, ADF, ADL, and organic matter *in vitro* digestibility (IVDOM). Although both GCA and SCA were significant for most quality traits, the quality traits differed from the agronomic characteristics in that variation attributable to GCA was generally greater than that of SCA, and environmental interactions were of lower relative magnitude. Narrow-sense heritabilities were greater than 80% for all quality traits. As long as maturity differences are accounted for, these findings regarding relatively high GCAs and heritabilities for quality traits are substantiated by most other studies involving similar quality measures.[205,254,260,267,268] The authors also concluded that selection for stover composition and digestibility should occur at the ensiling stage rather than at the later grain harvest stage. Gurrath et al.[269] supported this conclusion, but also noted that selection for forage yield could be conducted at either the ensiling or grain harvest stages.

TABLE 13.11
Neutral Detergent Fiber (NDF), *In Vitro* Digestible Dry Matter (IVDDM), and Digestion Kinetics of 45 Corn Inbreds at Early (Silking) and Late Harvest Dates (Physiological Maturity)

Inbred	Pedigree	Stem Internode[a] Early Harvest NDF (g/kg)	Stem Internode[a] Early Harvest IVDDM (g/kg)	Stem Internode[a] Late Harvest NDF (g/kg)	Stem Internode[a] Late Harvest IVDDM (g/kg)	Leaves[a] NDF (g/kg)	Leaves[a] IVDDM (g/kg)	Internode Digestion Kinetics[b] NDF (g/kg)	Internode Digestion Kinetics[b] Rate ($10^3 \times h^{-1}$)	Internode Digestion Kinetics[b] Extent (g/kg NDF)
B14A	BSSS C0	652	558	625	508	572	627	638	49	567
B14Ao2	BSSS C0	665	504	695	383	563	624	662	37	628
B37	BSSS C0	618	612	693	509	579	617	608	56	607
B52	MR164	642	504	691	430	589	632	643	40	577
B57	Midland	531	706	671	575	569	676	538	63	711
B64	41.2504B × B143	622	456	700	417	578	616	618	37	566
B68	41.2504B × B143	652	567	615	509	559	645	651	48	617
B73	BS13(HT) C5	604	613	689	454	560	632	584	61	578
B73o2	BS13(HT) C5o2	539	678	683	417	552	647	558	74	676
B75	BSCB3-92	612	595	606	580	582	612	604	50	556
B76	C131A × B372	582	659	681	522	557	614	573	60	613
B77	BS11(FR) C0–51	511	706	690	492	570	624	513	73	681
B78	BS13(HT)C6–5	556	655	685	495	578	628	564	64	637
B79	BS10(FR) C0-98	620	569	649	468	582	613	628	61	562
B84	BS13(HT) C7	588	625	688	443	575	631	588	59	595
B86	B52 × Oh43	565	525	623	481	602	586	546	42	582
B87	BS22 (H99)	573	672	686	519	582	614	590	55	657
B88	BS6(RC) C2	610	635	685	461	595	635	606	54	611
B89	BSSS(R) C7	635	594	775	372	575	645	636	47	600
B90	BSCB1(R) C7	624	636	622	535	582	621	612	54	631
B91	BSCB1(R) C8	575	655	718	417	594	630	592	56	656
B93	B70 × H992	545	680	643	565	523	636	546	58	616
B94	BSSS(R) C8	578	642	647	532	558	646	585	55	630
bm1/bm1	Genetic stock	574	669	707	537	543	665	544	69	735
bm2/bm2	Genetic stock	489	663	719	423	563	662	510	63	716

continued

TABLE 13.11 (CONTINUED)
Neutral Detergent Fiber (NDF), *In Vitro* Digestible Dry Matter (IVDDM), and Digestion Kinetics of 45 Corn Inbreds at Early (Silking) and Late Harvest Dates (Physiological Maturity)

		Stem Internode[a]				Leaves[a]		Internode Digestion Kinetics[b]		
		Early Harvest		Late Harvest						
Inbred	Pedigree	NDF (g/kg)	IVDDM (g/kg)	NDF (g/kg)	IVDDM (g/kg)	NDF (g/kg)	IVDDM (g/kg)	NDF (g/kg)	Rate ($10^3 \times h^{-1}$)	Extent (g/kg NDF)
BS16(V) C2-1bm	BS16 (V) C2-75-1-1bm	586	584	638	484	577	649	579	51	593
L289	Lancaster Sure Crop	616	567	757	300	552	583	633	45	596
L317	Lancaster Sure Crop	508	624	652	509	550	621	519	64	573
LAN232	Lancaster Sure Crop	588	665	711	499	569	580	578	60	668
LAN496	Lancaster Sure Crop	616	533	722	290	517	637	622	45	525
Mo17	CI187-2 × C103	563	674	578	592	571	627	574	71	663
N7A	Oh7/BSSS	580	682	752	523	572	641	603	54	642
NC252	B732 × Pa91	562	650	707	396	552	634	576	62	625
NC254	B732 × Pa91	567	644	719	424	546	670	576	55	648
NC256	B732 × Pa91	607	608	681	454	542	646	590	59	585
NC258	(NcNair14 × 18)^2 × [(NC248 × NC246) × C103]	588	641	551	597	587	622	587	62	687
NC262	McNair14 × 18	604	612	746	375	593	584	606	51	605
NC264	SC764 × Gaspe	616	623	784	334	572	596	578	62	645
NC266	NC250 × B732	593	649	647	521	575	643	593	62	598
NC268	NC250 × B372	583	623	676	486	569	663	578	58	602
NC270	B73 × NC250	602	581	658	447	564	639	593	60	593
NC272	Eto Blanco × B73G2	569	632	711	391	565	617	556	54	617
R225	BS10 C4	548	641	629	517	598	647	544	71	614
R226	BS10 C4	554	596	732	377	572	644	550	67	620
R227	RSSSC	498	692	630	543	561	641	497	77	659
Mean		584	620	679	469	569	630	584	57	621
Maximum		665	706	784	597	602	676	662	77	735
Minimum		489	456	551	290	517	580	497	37	525
LSD(0.05)		16	31	35	46	16	20	29	8	31

[a] Data from Lundvall et al.[263] Early and late-harvest NDF and late-harvest IVDDM adjusted by covariance analysis with silking date. Lowermost two above-ground elongated internodes sampled.
[b] Data from Jung et al.[264] Lowermost two above-ground elongated internodes sampled.

In France, Barrière et al.[205] reported high heritabilities for quality traits; broad-sense heritabilities for dry matter digestibility in two *bm3* synthetics were 53 and 71%. They further noted that the multivariate heritability of Gallais et al.,[270] which takes into account the colinearity of many quality and maturity traits, provided higher heritability estimates of 66 and 78%.

Geiger et al.[60,271] and Seitz et al.[272] used an incomplete factorial mating among a similar set of 11 flint and 11 dent lines. These studies provided combining abilities and genotypic correlations between inbred lines and derived hybrids for crude protein and metabolizable energy yield and content (MEY and MEC, respectively, using the procedures of Menke et al.[38]). Estimates of SCA were significantly greater than zero, but of minor magnitude relative to GCA for all traits but grain yield and forage MEY. They found a significant correlation between inbred composition and corresponding GCA for crude protein concentration on the basis of both stover and total forage, supporting previous research[273,274] that protein concentration is highly heritable. *Per se* MEC for inbreds was correlated with GCA for the flint inbreds for both stover and whole-plant forage. For dent inbreds, the correlation was significant for only whole-plant forage. Similar conclusions were reached by Gurrath et al.,[269] who, using the same set of inbreds and hybrids as Dhillon et al.,[57,165] calculated the correlation of inbred with derived-hybrid stover characteristics when harvested at the ensiling stage. Correlations for IVDOM, NDF, and ADL were all highly significant ($r = 0.78$, 0.71, 0.81, respectively). Based on these results, indirect selection for forage quality characteristics may be as efficient as direct selection for GCA.

In the Netherlands, variation in forage digestibilities was noted in the late 1970s,[275] and considerable attention has been devoted to determining the appropriate quality characteristic for selection. Deinum and Bakker[65] evaluated a collection of commercially adapted hybrids for whole-plant and stover characteristics, including *in vitro* organic matter digestibility and CWC. Whole-plant forage digestibility was highly correlated with digestibility of CWC ($r > 0.8$), whereas percent ear was not. This was confirmed by Deinum[256] using a larger set of hybrids collected from various European trials. Although ear percentage and cell wall concentration of the stover may be positively correlated, at least the digestibility of CWC seems independent of grain filling. Deinum and Bakker[65] calculated that whole-plant digestibility could be increased by 10 g/kg by either one of two methods: (1) increasing ear percentage by 6 to 8%, or (2) increasing CWD by 20 to 30 g/kg.

Dolstra and Medema[276] measured stalk digestibility in ten single-cross hybrids and found that heritability of CWD exceeded that of whole-plant digestibility (77 vs. 64%), suggesting that selection for stover digestibility, and particularly CWD, may be more effective than selection at the whole-plant level. Dolstra et al.[277] evaluated a set of 44 S_2 lines derived from four flint and five dent single-cross hybrids differing greatly for whole-plant digestibility. They again observed that digestibility of CWC was the most important discriminating factor for selection of improved stover and whole-plant nutritive value. In addition, CWD seems to be less effected by plant maturity than other quality traits.[165,278,279]

Determining digestibilities using the standard or modified protocols of Tilley and Terry[35] requires access to fistulated cattle and can therefore be somewhat cumbersome. Because the extent of cell wall lignification is strongly associated with CWD,[19] assays of lignin concentration may be adequate for selection. Highly significant correlations between lignin concentration and whole-plant digestibility have been reported (e.g., $r = -0.81$[254] and –0.96[165]), and the lignin concentration in inbreds is correlated with digestibility of derived hybrids.[165,269] As noted by Roth et al.,[254] however, the absolute value of lignin concentration may have little physiological meaning. The degree to which lignin complexes with cellulose and thereby reduces its digestibility is likely to be more important than the absolute lignin concentration. It should be expected that variation in digestibility will occur even within relatively narrow ranges of lignin concentration.

There are few reports dealing with recurrent selection programs dedicated to increasing nutritive value of corn silage. As described earlier, S_1 family recurrent selection has been used to modify fiber and lignin concentrations of stover tissue in the WFSIHI and WFISILO populations, and compositional characteristics of selected inbreds derived from breeding populations were expressed

when they were evaluated in crosses.[67,121,134,139,140] However, the corn populations used in these studies were genetically broad-based, and the range in quality attributes may have been greater than what is available in more agronomically acceptable germplasm. The only reported attempt to improve both forage yield and quality using recurrent selection techniques is that of Hunter[88] who used S_1 family selection in two populations, CG Syn A and Wigor. CG Syn A was an early Canadian dent derived from North American germplasm, and Wigor was an early flint population from Europe. Five separate selection programs, each with a single selection criterion, were applied to CG Syn A: (1) high whole-plant yield; (2) high whole-plant *in vitro* digestibility; (3) low whole-plant *in vitro* digestibility; (4) high yield of digestible dry matter; and (5) high grain yield. Only criteria 4 and 5 were applied to Wigor. Results after two cycles are presented in Table 13.12, where cycles 0, 1, and 2 are compared for silage performance as evaluated in 1984. When CG Syn A was selected for high grain yield there was little improvement for whole-plant forage yield, and no improvement in digestibility. The greatest improvement in whole-plant yield for this population occurred as the result of S_1 selection for whole-plant yield or digestible dry matter yield. Neither selection criteria produced much improvement in whole-plant digestibility. Selection for either high or low digestibility did not greatly affect dry matter yield, and only the latter criteria seemed effective at modifying (in this case lowering) whole-plant digestibility. The lower-yielding Wigor population was more amenable to selection for either grain or whole-plant yield, and selection for whole-plant yield was very effective at increasing whole-plant yield from 6.0 to 11.6 Mg/ha. There were, however, no associated changes in digestibility. The only conclusion was that S_1 selection for whole-plant yield was more effective if based on direct rather than indirect criteria, i.e., whole-plant yield instead of grain yield. The lack of any improvement in nutritive value as a result of selection for digestibility was somewhat disappointing given the preponderance of evidence that quality traits seem highly heritable.

Mapping of quantitative trait loci (QTL) may identify genomic regions of particular importance for silage quality. Lübberstadt et al.[64,280] developed 380 F_3 families from a cross of two elite flint inbred parents, and these were crossed to two diverse dent inbred testers. A large sample (345) of the F_2 individuals from which the F_3 families were derived were also genotyped using 89 restriction fragment length polymorphisms (RFLPs). Traits evaluated included dry matter yield, starch concentration, IVDOM, ADF, MEC, and crude protein. Heritabilities were higher for dry matter yield and starch concentration (>0.64), but relatively lower for IVDOM, ADF, and MEC (<0.45). Crude protein heritabilities were between 0.37 and 0.59. Between four and ten QTL were detected in each testcross. Results were consistent across testcrosses for crude protein, but not dry matter yield, starch concentration, IVDOM, ADF, and MEC. As expected, the digestibility traits IVDOM, ADF,

TABLE 13.12
Whole-Plant Yield and *In Vitro* Digestible Dry Matter (IVDDM) Resulting from Three Cycles of S_1 Recurrent Selection for Several Selection Criteria in Two Corn Populations

		Whole-plant yield (Mg/ha)			Whole-plant IVDDM (g/kg)		
Population	Selection criteria	C0	C1	C2	C0	C1	C2
CG Syn A	Grain yield	10.7	10.9	11.0	713	699	708
	Whole-plant DM yield	10.7	10.9	12.4	713	701	716
	Whole-plant digestible DM yield	10.7	11.2	12.8	713	707	713
	High IVDDM	10.7	10.5	11.1	713	694	714
	Low IVDDM	10.7	11.6	11.7	713	711	694
Wigor	Grain yield	6.0	6.6	9.1	720	724	711
	Whole-plant digestible DM yield	6.0	8.9	11.6	720	720	722

Source: Data from Hunter, R. B.[88]

and MEC were highly correlated with each other. With two exceptions, no single putative QTL contributed more than 10% of the trait variation in both testcrosses. The two exceptions were a dry matter yield QTL on chromosome 1 (R^2 = 15.5 and 19.5%, in testcross 1 and 2, respectively) and an IVDOM QTL on chromosome 3 (R^2 = 13.0 and 11.4%, in testcross 1 and 2, respectively). Digenic epistasis was significant for dry matter yield at several QTL, but it was less important for quality QTL. As emphasized by Melchinger et al.,[281] for those traits with moderate or low heritabilities, where marker-assisted selection would be most efficient, the chances of QTL detection are fairly low at the sample sizes that can be handled practically. Therefore, unless QTLs are found to be consistent across populations, it may not be particularly efficient to use them in selection. In a subsequent study, Lübberstedt et al.[282] therefore evaluated the consistency of QTL for three additional sets derived from different populations involving a total of four flint inbreds. All lines derived from these four mapping populations were crossed to the same dent inbred tester. QTL results were not consistent among populations, and the authors recommended that separate QTL analyses were needed for each individual population before making any attempt to use marker assisted selection. At this time, marker-assisted selection may be practical for only a few elite populations.

V. POTENTIAL VALUE

It is exceedingly difficult to put a dollar value on projected improvements in any measure of nutritive value. Most current studies focus only on laboratory estimates of quality. True assessments involving reliable animal performance trials and economic analyses evaluating the extent of variation encountered within species such as corn are not available. Even if appropriate models can be agreed upon, estimates of economic value for quality improvements will vary depending on crop productivity, animal species, and the many production systems employed, and it is not the intent of the authors to thoroughly examine such issues, but rather to draw tentative and rather limited conclusions about potential economic returns attributable to quality improvements.

One simple method commonly used to assign monetary value to quality improvements is to equate expected improvement in either *in vitro* digestibility, or expected increases in digestibility resulting from reduced NDF or ADF, with increased TDN. If a silage has lower fiber concentrations, it should contain more energy because almost all non-fiber fractions are rapidly digestible, and therefore less energy supplementation is needed. Various assumptions are made regarding (1) level of silage used in the ration, (2) dry matter intake, and (3) cost of supplemental TDN. Assuming improvements in whole-plant digestibility of 25 to 50 g/kg are possible without sacrifice of other agronomic characteristics, then for a dairy producer under current economic conditions and production schemes in Wisconsin, the value of increased TDN translates, over a 150-day lactation period, to approximately $7.50 to $15 per cow or $750 to $1500 per 100-cow herd (R. D. Shaver, Dep. of Dairy Science, Univ. of Wisconsin-Madison, pers. commun., 1992). This type of approach is used by several seed companies to promote their silage varieties, although some may claim additional benefit to increased protein concentrations.

Carter et al.[253] used a variation of this approach for dairy cattle by formulating hypothetical rations at equal milk production for a typical high-producing herd, and then calculated expected savings in daily feed costs due to the reduced use of energy supplements with a high-quality corn silage. Using the ration-balancing method developed for Wisconsin dairy producers,[283] and assuming the silage makes up 25% of the diet, two types of comparisons were presented (reflecting the range in nutritive values presented by Carter et al.,[253] in Table 13.9). The first compared a silage variety with relatively high quality (410 g/kg whole-plant NDF, 210 g/kg ADF) and average grain yield with hybrids with average forage quality (450 g/kg whole-plant NDF, 250 g/kg ADF). In this comparison, the higher-quality silage provided an additional return of approximately $84 per hectare. The second involved a comparison of a hybrid with above-average forage yield (49.3 Mg/ha at 350 g/kg dry matter) with a hybrid of average yield (44.8 Mg/ha at 350 g/kg dry matter), both having average quality. In this case the higher-yielding hybrid conferred an added return of $123

per hectare. This analysis demonstrates the relative economic benefits on improvements of yield and quality given the relatively narrow ranges in quality measures provided by the data of Carter et al.[253] and avoids the pitfalls inherent in comparing silages representing the high and low extremes in nutritive value. Nonetheless, it may be somewhat conservative. As the authors emphasize, the amount of silage in the typical Wisconsin ration (25%) is low compared with other dairy regions, such as the northeastern U.S., and energy replacement value of high-quality hybrids would increase substantially with a greater proportion of silage in the diet. It has also been noted by Deinum and Bakker[65] and Deinum[256] that the variation among hybrids in cell wall composition (e.g., degree of lignification) as reflected in their measures of CWD should have pronounced effect on feed intake, perhaps greater than that due to differences in energy value. At a given level of fiber intake, any increase in fiber digestibility may provide an additional increase in milk production.

A method used by Lauer et al.[46] to evaluate the economic trade-off among commercial hybrids is the performance indices of milk per megagram (kilogram of milk per megagram of corn forage) and milk per hectare (kilogram of milk per hectare of corn forage).[284] Milk per megagram is predicted using *in vitro* true digestibility, crude protein, and neutral detergent fiber values from equations for feed intake and animal requirements for a standard dairy cow with 613 kg of body weight producing 36 kg of milk per day at 3.8% fat. Milk per hectare is the product of milk per megagram and dry matter yield of corn forage. One limitation of this method is that it does not fully account for the variation in CWD among hybrids.

There may be more obvious benefit to silage quality improvements for beef cattle. Hunt et al.[285] compared silages from two Pioneer brand hybrids (3377 and 3389) that had similar grain percentages, but varied in whole-plant digestibility, in steer calf feeding trials. Steers fed the higher quality silage (3377) had 8% greater daily gain, and 10% greater feed efficiency than those fed the 3389 silage. Because the two hybrids were of equal whole-plant yielding potential, a producer could apparently obtain increased feed efficiency without sacrifice. The authors stated that given the reported differences in feed efficiency and current prices for feeder calves and feed, the relative quality advantage of 3377 over 3389 would be worth $778 per hectare (C. Hunt, Univ. of Idaho and W. Kezar, Pioneer Hi-Bred, pers. commun., 1991). If the approach taken by Carter et al.[253] is used, i.e., comparing rations on the basis of energy supplement savings, and assuming that calves were fed rations containing at least 50% silage, the projected benefit due to feeding 3377 silage would be $193 per hectare. The high value on a hectare basis reflects, in part, the high forage yields possible under irrigated production.

These examples, although overly dependent on relatively simple models that ignore the extreme variation in crop and livestock systems, do indicate that there is potential economic benefit for producers should nutritionally improved silages become available. Currently several U.S. companies are assessing nutritional characteristics of currently available hybrids and then using such information primarily for marketing. This approach seems effective in attracting attention of producers, but the success of this tactic on increasing seed sales or market share is not generally known.

VI. FUTURE BREEDING POTENTIAL OF SILAGE CORN

Geneticists and breeders are beginning to focus efforts on altering digestibility of slowly degraded tissues in the leaf blade, sheath, and stem. Enzymes specifically involved in formation of lignin, such as phenylalanine ammonia-lyase, cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), or *O*-methlytransferase, can be regulated by relatively simple compounds. There are also a number of peroxidases and oxidases that react with lignin precursors during the formation of the lignin polymer. The lignin metabolic pathway is highly complex, and there are probably a large number of ways to modify lignin concentration and composition. Genetic engineering techniques that would allow transformation and precise tissue-specific expression of inhibitory compounds (e.g., antisense RNA from cloned genes involved in lignin biosynthesis) may provide the molecular tools needed to better regulate cell wall composition.[177,286–294] However, there is

considerable metabolic plasticity involved in plant lignification, and attempts to down regulate enzymes involved in lignification can have unintended consequences. Ralph et al.[295] used homologous antisense constructs to down regulate CAD and CCR in tobacco. While overall lignin content was reduced in antisense-CCR tobacco, the results from antisense CAD tobacco were unexpected, indicating that the results of such down regulation are enzyme dependent and may not lead to reduced lignification. The antisense-CAD tobacco did contain fewer coniferyl and sinapyl alcohol-derived units, but also showed compensatory increases in benzaldehydes and cinnamaldehydes. Falkner et al.[211] and Argillier et al. [296] also showed that the relationships between cell wall digestibility and probable lignin cross-linking sites such as arabinose, as well as the cross-linking units involved such as esterified or etherified ferulic acid, may not yet be well enough understood to be completely predictive. Plants seem to have evolved the ability to circumvent genetic or metabolic obstacles to making lignin by utilizing a variety of alternative phenols and cross-linking strategies, which is quite sensible for such an important polymer.[297]

In the near term, selection for improved forage yield and quality will probably involve conventional selection techniques for yield and maturity, coupled with inbred assessment for quality traits. Recurrent selection and conventional pedigree systems using both inbred and outcrossed progenies would undoubtedly improve nutritive value. Breeders have two complementary approaches to increasing total energy content: (1) increase proportion of grain, and (2) increase digestibility of stover. In an economic assessment of breeding strategies, Utz et al.[61] found that under moderate animal performance levels, selection for either grain yield at silage harvest or simultaneous selection for forage DM yield and ear percentage would provide the most economical means of improving yield and quality. However, when breeding silage hybrids for feeding to high-performance animals, one parameter related to digestibility of CWC should be included.

Stover NDF and CWD were the two quality-related traits that provided the greatest expected gains from selection in the variance component evaluation of Almirall et al.[68] Stover CWD has received most attention because it is usually more highly correlated with whole-plant digestibility than is grain content.[54,65,67,121] Stover CWD is highly correlated with whole-plant digestibility because (1) ruminants derive a major proportion of dietary energy by fermenting structural carbohydrates in the cell wall portion of the plant, and (2) grain is low in NDF and nearly completely digested. Even for hybrids with similar grain-to-stover ratios performance trials with dairy cows have demonstrated considerable hybrid to hybrid variation, suggesting that digestibility of NDF is important. Many now agree that including stover CWD in a silage hybrid selection program has potential to improve nutritive value of corn silage.

Maturity and choice of plant tissue for at sampling, as well as stage of testing during breeding (i.e., inbred vs. hybrid), will also influence selection efficiency. Planting and harvest dates can be modified to account for maturity differences among hybrids or inbreds, but choice of plant tissue is more problematic. While stover analysis provides the most direct estimate of fiber content and composition, it is still not clear whether stover analysis of inbreds provides the most efficient quality screen for breeders. Inbreds are quite sensitive to environmental stress, and they vary considerably for ear content. These factors will be confounded with true genetic differences in nutritive value when inbreds are compared for stover composition.[155,156,161] There are several ways to address this concern. Corn breeders might evaluate inbreds for only those traits, such as CWD, that are not greatly affected by ear-fill and maturity effects.[268,277,279] The most conservative approach would be to forego inbred evaluations entirely and evaluate quality only on a whole-plant basis for tested hybrids. Combined selection methods such as those described by Gouesnard[298] and others,[270,299–301] where inbred progenies would be selected primarily for stable quality characteristics while outcrossed progenies would be selected with more emphasis on whole-plant yield and maturity, seem especially appropriate.

Corn breeders in the U.S. have sufficient information to suggest that breeding for high-quality forage corn has great potential. Increasing interest at the corporate and farm levels has focused attention on the merits of creating novel high-quality germplasm, and the new silage hybrids released

in recent years have achieved some success in the seed market. New genetic technologies will undoubtedly provide alternative avenues to improve quality in the future. There are no adapted germplasm sources specific for silage hybrids at this time other than those developed by incorporating single genes known to affect quality, such as *bm3,* into current inbreds. It remains to be seen whether corn breeders in the U.S. will adopt a long-term germplasm improvement approach such as that used in the past for grain hybrids.

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14 Temperate Corn — Background, Behavior, and Breeding

Forrest Troyer

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I. INTRODUCTION

A. Foreword

This is a revision of my earlier chapter "Breeding Early Corn," in the first edition of this book. I have solicited and received helpful suggestions for improvement of the original. About 90% of temperate corn hybrids are season limited, which is a good definition of early temperate corn. Background includes introduction and evolution; behavior includes form and function; and breeding includes inbreds and hybrids.

My approach to corn breeding is to study the crop to help understand its workings, and to use that knowledge to breed more productive corn plants. Go to the field to observe the crop. Speculate and make predictions to form a tentative hypothesis. Often the hypothesis has few facts and much speculation initially, then more facts are gathered that validate the hypothesis or suggest a new one. This approach constructively builds useful corn breeding objectives from personal experiences. My experiences have usually involved only a few principles. They include relationships between corn and its background (evolution), relationships between corn and its behavior (form and function), and how these relationships affect breeding superior inbreds and hybrids. I have included some geography and some climatology affecting these relationships.

My purpose is to help the reader learn about corn breeding from experiences that have helped me. Stated differently, these are things I wish I had known sooner. I will cover a broad area and provide enough references for further study. I recommend *Corn and Corn Improvement,* edited by Drs. George F. Sprague and John W. Dudley; it is an excellent general corn reference and source of many references. I also recommend *Principles of Plant Breeding, second edition,* by Dr. Robert W. Allard; it delivers what the title promises. My treatment features commercial application, and will emphasize temperate corn, most of which is usually grown where the length of season is limiting. This narrowing of scope is useful because some general source-sink relationships then exist.

B. Philosophy

I believe it helps to have a high regard for the organism with which one is working. I try to understand what the plant is experiencing to learn how it works. The ultimate result is to anticipate how an inbred or a hybrid will react in different environments. Certainly observation and sympathy for the plant have helped me develop useful selection screens to aid phenotypic selection during inbreeding.

Education, experience, and insight influence a corn breeder's decisions. Education should be a never-ending process. Some researchers become obsolete because they quit learning soon after they quit going to school. Reading books, reading periodicals, and attending meetings are pertinent and helpful. Learning from others' experiences greatly expands available knowledge. Personal corn breeding experience comes a year at a time from yield tests and comes twice a year from nurseries. Experience takes time. Years are the major component of corn genotype-by-environment interaction because seasons differ. The good part of experience is to let the plants show you what is needed to help them. Experience provides a corn breeder's best

education, because interactions and their effects can be anticipated to reduce risk. Cumulative experience results in improved insight. One's insight and understanding are as good as one's experience and education.

Commercial corn breeders are part of an organization, and as such they must help their organization thrive by meeting objectives and expectations. Because corn breeders fulfill a creative function, association with key users of their creations is helpful. Once on the job, important learning experiences for setting corn-breeding objectives come from association with the farmer, with sales and marketing personnel, with production and foundation seed personnel, and with corn end users. Your job is to give the farmer what he wants. Educating the farmer is someone else's job. The single thing that has helped me the most is to learn what the farmer is buying, then provide something that is similar, but better, for a recognizable trait (usually more yield and more stress tolerance).

Some breeders fail because they underestimate the farmer. They think they know what the farmer needs better than the farmer knows. The farmer knows exactly what he needs, and he's buying it. Whenever farmers are doing something different than I expect, I learn from them again. Sales people are on the firing line, constantly facing rejection. As a group, they furnish excellent second-person feedback from the farmer. Give them what they want and they will sell it. Marketing people are trained to analyze trends, surveys, statistics, etc. to help your company sell corn. Help the marketing people, and they will help you. It is an important step forward when sales, marketing, production, and research people are truly trying to help each other. Production and foundation seed personnel are particularly helpful in spotting traits that limit yield or complicate seed harvest. Ultimately, corn breeders serve farmers and end users. Time spent with them is time well spent.

The U.S. has excess corn production capacity. Total production has increased due to increased yield per acre, but acreage could also increase if more demand existed. About three fifths of the U.S. corn crop (58%) is used for feeding livestock, about a fifth (22%) is currently used for export, and a fifth (20%) is used for food, alcohol, and industrial uses. Final use of U.S. corn, including export for feed and including industrial users by products for feed, totals four fifths (81%) for animal feed.[1] Increasing yield to reduce the cost of production is the best way for corn breeders to increase use of corn. Industrial users want the least expensive source of starch. Another way to help end users is to reduce the number of broken kernels.[2]

C. Inheritance of Maturity

The inheritance of maturity in corn is governed quantitatively. In general, the genes act in an additive manner, as if many genes with small and equal effects were involved. Heterosis complicates studying the inheritance of maturity because vigor hastens maturity, and conversely, inbreeding decreases vigor and delays maturity. On the average, heterosis affects flowering time about 10%. If two unrelated inbreds average 1500 growing degree units (GDUs) to flower, the hybrid of these two inbreds will require 1350 GDUs to flower; i.e., 150 GDUs (10% of 1500) less than the average for the two parent inbreds.

Day length response of corn affects flowering time (Table 14.1). Reports exist that some earlier corns grown in longer-season areas are insensitive to day length, but I believe all corn is sensitive to day length if properly tested where it is adapted. A proper test separates day length and temperature effects. Planting must be done in the spring before the summer solstice. The corn should be planted at two or more dates and at two or more latitudes with comparisons for GDUs to flower and for plant height. Corn planted at the same latitude on later dates (providing longer days and warmer temperatures) flowers later and grows taller than the corn planted at earlier dates. Corn planted on the same date at higher latitudes (providing longer days and cooler temperatures) flowers later and grows taller than the corn planted at the lower latitudes. In these two experiments both temperature and day length are changing. In the first experiment (with later dates) temperature

TABLE 14.1
Daylength for Summer Solstice (Longest Day) for Certain Latitudes and Mean June (North Latitude) or Mean December (South Latitude) Temperature (°C) for Some Cities Near Those Latitudes

Latitude	Daylength		
(degrees)	Hours	Min	Cities (June or December mean temp. °C)[2]
90	24	00	North Pole (–21), South Pole (–30)
60	18	53	Anchorage (14), Uranium City (11), Oslo (18), Helsinki (14), St. Petersburg (17)
55	17	23	Saskatoon (17), Hamburg (16), Copenhagen (16), Smolensk (20), Moscow (17), Kazan (21), Omsk (17), Novosibirsk (13)
50	16	23	Vancouver (17), Lethbridge (17), Winnipeg (19), London G.B. (16), Paris (17), Brussels (17), Frankfort (17), Leipzeig (18), Prague (17), Vienna (18), Wroclaw (18), Krakow (18), Warsaw (18), Kiev (18), Kharkov (19)
45	15	37	Watertown, SD (24), Minneapolis/St. Paul (21), Green Bay (20), London, Canada (21), Ottawa (20), Montreal (20), Bordeaux (21), Lyon (21), Milan (24), Zagreb (21), Budapest (21), Belgrade (21), Sofia (21), Bucharest (23), Odessa (21), Kransnodar (22), Harbin (23), Sapporo (20)
40	15	01	Denver (20), Lincoln (21), Kansas City (24), Des Moines (22), Decatur (23), Indianapolis (23), Columbus (24), Philadelphia (23), Madrid (23), Naples (24), Athens (26), Ankara (23), Beijing (27), Seoul (24)
35	14	31	Los Angeles (22), Little Rock (26), Raleigh (25), Baghdad (32), Kabul (24), Sian (25), Tokyo (24), Santiago (26), Buenos Aires (23)
0	12	07	Quito (14), Macapa (27), Leopoldville (21), Nairobi (25), Singapore (27)

[a] Weather records courtesy of Dr. Wayne M. Wendland, State Climatologist, Illinois State Weather Survey, Urbana, IL, 61801.

is warming while in the second experiment (with higher latitudes) temperature is cooling. Therefore, the later flowering and the taller plant height are due to longer day length and not due to temperature effects. Furthermore, corn planted on the same date at lower latitudes (providing shorter days and warmer temperatures) flowers sooner and grows shorter than corn planted at higher latitudes. Short-day day length reaction limits north to south adaptation. Fewer maturity zones would exist if the day length reaction were reversed; i.e., if corn had long-day day length reaction.

Flowering dates generally are compressed in winter nurseries compared with summer nurseries. Flowering dates are spread more in summer nurseries as one goes to shorter-season areas. These are primarily temperature effects; good winter nurseries are warmer than short-season summer nurseries, and shorter-season areas generally get cooler (fewer GDUs) as they get shorter.

Tropical corn grows very tall in temperate areas. It flowers near the fall equinox when day length becomes short enough to trigger short-day day length reaction. Tropical corn flowers with

fewer GDUs in tropical winter nurseries where the days are shorter. A tropical variety that flowers late in the tropics and flowers late in a tropical winter nursery is virtually hopeless for temperate use.[3]

D. Maturity Designations

Adapted hybrids flower late enough to provide adequate plant size, yet early enough to complete or nearly complete grain filling in an average length season, according to Troyer and Brown.[4] Maturity zones indicate where hybrids of different maturity groups should be grown in a normal or average season. They match area maturity (season warmth and length) with hybrid maturity (flowering date and harvest moisture). Full-season hybrids usually yield more than earlier hybrids in geographic areas where season length is limiting. Maturity zones are based on accumulated GDUs during the frost-free period:

$$\text{GDU} = \frac{\text{Max. temp. (ð30°C)} + \text{Min. temp. (Š10°C)}}{2} - 10\text{°C}$$

When calculating GDUs, daily maximum temperatures above 30°C are entered as 30 and minimum temperatures below 10°C are entered as 10. GDUs and dates of frost are affected by elevation, by nearness to large bodies of water, by ocean currents, by prevailing winds, by soil types, and perhaps by other factors. Corn wastes GDUs in warmer-than-average seasons and more efficiently uses GDUs in cooler-than-average seasons because the temperature-growth relationship is not a perfectly straight line.

Minnesota's corn maturity law was enacted in 1939 and revised in 1961. It established relative maturity zones and hybrid maturity classifications.[5,6] Hybrids must be registered and bags labeled with official relative maturity (RM) ratings to be sold in Minnesota. The Minnesota Agricultural Experiment Station grows new commercial hybrids along with adapted and nonadapted check hybrids in the appropriate maturity zones. Tests are harvested near the middle of the harvesting season for each area. Check hybrid RM is regressed on harvest moisture. New hybrids are assigned RMs based on their harvest moisture. Originally, day ratings were defined as the number of days from emergence to physiological maturity (safe from frost) at St. Paul in an average season, but this statement was soon dropped. Emphasis is now placed on the difference between hybrid ratings (days RM). The farmer considers 5 days RM a significant difference in maturity in short-season areas.

Dr. Karl Knittle, Mr. Doug Hill of St. Louis, and I modified University of Minnesota maturity zones to fit the major corn growing area of the U.S. (Figure 14.1). The map features narrower zones at higher latitudes where maturity is more critical and wider zones at lower latitudes where it is not. The modified and Minnesota relative maturity regressions cross at 88 RM; the modified map provides more ratings (hybrids) for smaller geographical areas where they are needed in shorter season areas and fewer ratings for larger geographical areas where maturity is less critical in longer season areas. More GDUs (higher temperatures) can be detrimental. In central and southern Texas, earlier hybrids (115–120 RM) are grown to avoid high nighttime temperatures that occur later in the season.

Ontario maturity zones were developed at Guelph University.[7] Corn growing in Southern Ontario is greatly affected by its proximity to the Great Lakes, by cloudiness, by elevation, and by soil type. Most farmers in Ontario adhere closely to the recommended Ontario corn heat unit (OCHU) maturity zones.

The Food and Agricultural Organization (FAO) developed European maturity zones in 1954.[8,9] FAO used American hybrids of known Minnesota relative maturity as checks. Many European countries require that hybrids for sale have an FAO maturity rating attached. The USDA has used an AES (Agricultural Experiment Station) number that is comparable to the FAO number. European heat units are calculated with a lower average minimum temperature for growth:

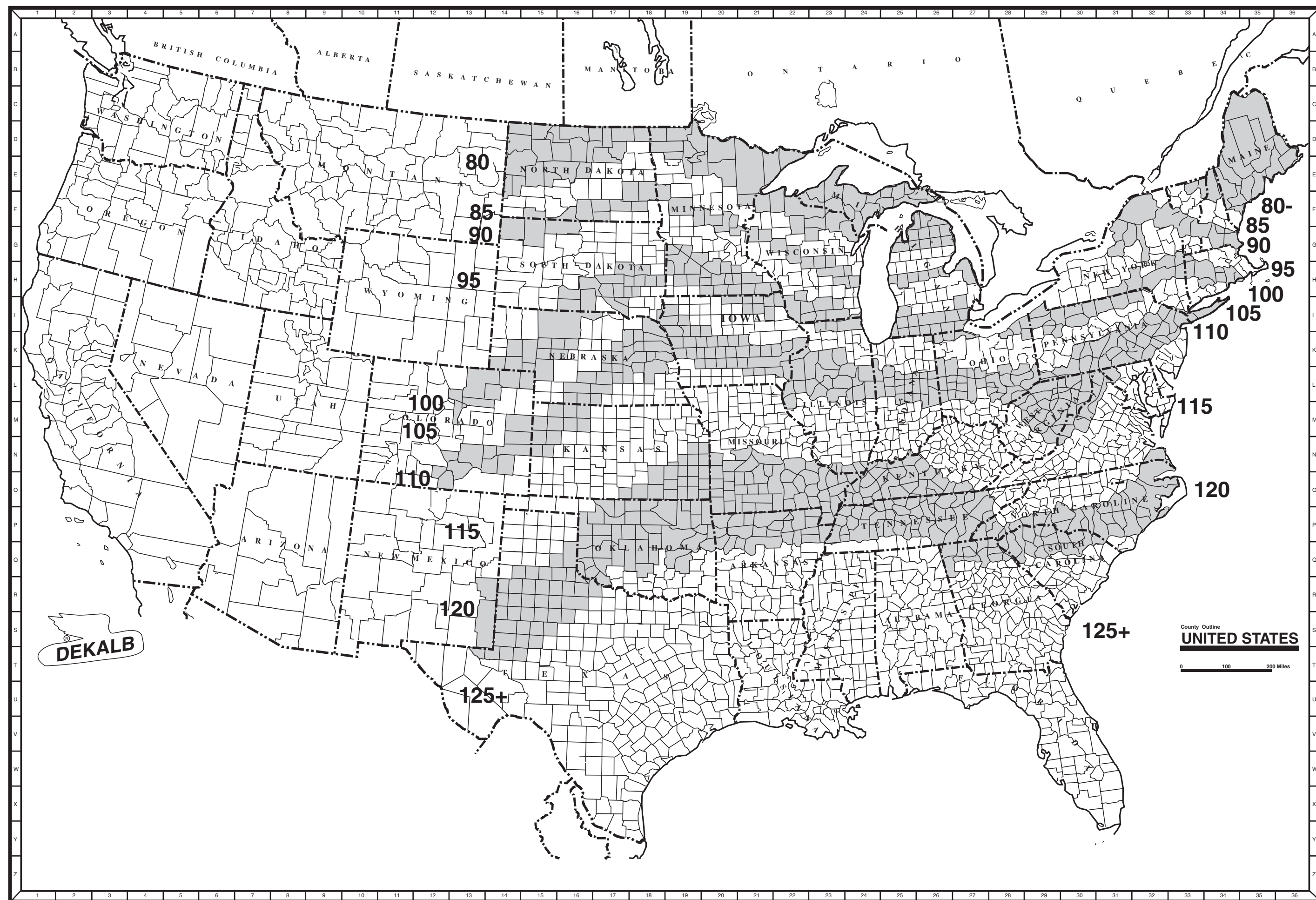


FIGURE 14.1 Map of the United States of America showing relative maturity zones for corn growing.

$$\text{GDUs} = \frac{\text{Max. temp. (ð30°C)} + \text{Min. temp. (Š6°C)}}{2} - 6°\text{C}$$

Some recent European studies suggest 8°C minimum-temperature base gives more consistent results over seasons than 6°C. Some European countries have altered the original FAO system. Germany, for example, divides the scale into smaller units between zones and hybrids. Comparisons of corn relative maturity equivalents are presented in Table 14.2.

E. Flowering Date and Drying Rate Effects on Harvest Maturity

When adapted corn is moved to a shorter-season area, length of kernel filling period is usually limiting. When adapted corn is moved to a longer-season area, plant size is usually limiting. Stated differently, shorter stature (earlier flowering) corn of an adapted RM is more likely to yield well when moved to a shorter-season area than will taller stature (later flowering) corn. And, conversely, taller stature (later flowering) corn of an adapted RM is more likely to yield well when moved to a longer-season area than will shorter stature (earlier flowering) corn. These effects are increased by short-day day length response as explained earlier.

Still another way to say it, earlier flowering hybrids of the same RM as later flowering hybrids may be used more successfully in areas with fewer GDUs, because earlier flowering starts the kernel filling period sooner. Later flowering hybrids of the same RM as earlier flowering hybrids may be used more successfully in areas with more GDUs, because later flowering causes larger plant size to allow more photosynthesis.

Faster drying hybrids of the same RM as slower drying hybrids allow later flowering and higher potential yield because their larger plants can produce more photosynthesis. These hybrids are handicapped in shorter- or cooler-than-average seasons because of a shortened kernel filling period. They are more likely to have chaffy, low test weight kernels.

F. Definition of Temperate and of Early Corn

Temperate corn is usually grown in the temperate areas (beyond 23.5° latitude). Most temperate corn (90%) is grown in areas where the seasons are usually limited by GDUs, by length of growing season, or by both. Corn limited by season is 115 RM (B73 × MO17) and earlier in North America during the past 25 years. Thirty-five years ago, later corns grown in the southern U.S. were sometimes limited by season length. Since that time, earlier corn has more generally been grown there. Southern farmers benefit by earlier planting of earlier corn so the corn flowers before midsummer heat, and by earlier harvest with higher prices. Both U.S. Corn Belt breeding progress and combine harvest have caused this change.

In Western Europe corn limited by season is 102 RM (A632 × A619) and earlier in France, and 85 RM (Dea) and earlier in Germany. The oceanic climate (Gulf Stream, North Sea, and Mediterranean Sea) generally provides a warmer and longer season than North America on a relative latitude basis (see Table 14.1, Table 14.11). Springs are cool in Western Europe, and late spring frosts are common. Falls in Western Europe have later frosts than in North America. The corn-growing season in France usually ends before frost when wheat-planting time comes in early October. Corn seasons in Spain are more like the southern United States where the growing season length is seldom limiting.

Eastern Europe has a continental climate; growing conditions are similar to the western U.S. Corn Belt, with 115 RM (B73 × MO17) as the latest corn commonly grown. Wheat is the most important crop in the Ukraine and in Russia; wheat-planting time signals time for corn harvest. Argentina has early corn in shorter-season growing areas south of Buenos Aires, where 102 RM (A619 × A632) and earlier corn has limited growing seasons. China has early corn in shorter-season growing areas north of Beijing, in northeast China, where 102 RM (A619 × A632) and earlier corn

TABLE 14.2
Corn Relative Maturity Rating Equivalents

DeKalb (U.S.) Relative Maturity (days)	Minnesota Relative Maturity (days)	U.S. Growing Degree Days (GDU)	Canadian Heat Units (OCHU)	FAO, AES Relative Maturity (units)
75	70	1650	2100	100
78	75	1750	2300	
82	80	1850	2500	200
86	85	1950	2600	
89	90	2050	2700	300
93	95	2150	2800	
96	100	2250	2900	400
100	105	2350	3200	
104	110	2450	3400	500
108	115	2250	3500	
111	120	2650	3700	600
114	125	2750	3900	
118	130	2850	4100	700
121	135	2950	4300	
125	140	3050	4500	800

has a limited growing season. Japan has early corn on the island of Hokkaido where Dea, a popular 85 RM hybrid, and earlier corns have limited growing seasons.

Early corn must grow faster and mature sooner under cooler conditions than later corn to produce mature kernels in a shorter season. Early corn is early because it is more vigorous. It grows faster, particularly in the spring when the weather is cool. The extremely early Northern flint varieties grow very fast in seedling and in juvenile plant stages.[10] Therefore, early corn flowers and reaches physiological maturity sooner than later corn. Late dent varieties and races from the southern U.S. and from Mexico grow very slowly in seedling and in juvenile plant stages. These corns evolved where they were typically planted late in the spring in warm soils. Religious taboos (chastity for the period from corn planting until emergence) encouraged good, warm seedbeds (later planting) during evolution.[11] Corn is now planted earlier in the southern U.S., when soils and weather are still cool. Aboriginal southern U.S. materials germinate poorly, grow slowly, and also often get northern leaf blight (*Helminthosporium turcicum*) to which they are generally susceptible.

G. Sources of Corn Germplasm

U.S. sources of corn germplasm include the National Seed Storage Laboratory at Fort Collins, Colorado. USDA Regional Plant Introduction Station at Ames, Iowa has early open-pollinated varieties from all over the world. U.S. Agricultural Experiment Stations at Urbana, Illinois; Lafayette, Indiana; Ames, Iowa; East Lansing, Michigan; St.Paul, Minnesota; Columbia, Missouri; Lincoln, Nebraska; Ithaca, New York; Fargo, North Dakota; Wooster, Ohio; State College, Pennsylvania; Brookings, South Dakota; and Madison, Wisconsin can provide released inbreds and hybrids. Canadian corn breeding efforts at Brandon, Manitoba; Morden, Manitoba; Guelph, Ontario; Harrow, Ontario; and Ottawa, Ontario can provide released inbreds and hybrids. Ag Alumni at Romney, IN; Mike Brayton Seeds at Ames, IA; Downing's at New Madison, OH; Glenn Foundation Seed at Blenheim, Ontario, Canada; Holden Foundation Seeds at Williamsburg, IA; Illinois Foundation Seed at Urbana, IL; PLS Genetics at Atlanta, IN; and Thurston, Inc. at Olivia, MN are foundation seed companies that have developed inbreds and hybrids.

Additional sources of corn germplasm include Slachtitelska Stanica, 930 11 Topolniky, Czech Republic; Station D'Amelioration des Plants, Route de Lyon, 63039 Clermont Ferrand, France; Station D'Amelioration des plants, 34060 Montpelier Cedex, France; Station D'Amelioration, 80200 Peronne, France; University of Hohenheim, Prof. Dr. W. G. Pollmer, D70599 Stuttgart, Germany; Hokkaido National Agricultural Experiment Station, 1 Hitsojigaoka Toyohia-ku, Sapporo 061–01, Japan; Plant Breeding Station, 63–743 Smolice, Poland; Agricultural Research Station, Str. Agriculturii 27, R.S. Romania; Vavilov Institute, 42 Herzen Str. 190000 St. Petersburg, Russia; and University of Zagreb, 4100 Zagreb, Yugoslavia.

H. Economic Importance of Temperate Corn

Temperate corn areas by country, by use, and by maturity are listed in Table 14.3. These data conform to the definition of temperate corn limited by season length. The early/late RM estimate may change over time due to new cropping practices and due to change in market share of new popular hybrids of different RM. Corncob mix (ground whole ears and husks) in northwestern Europe is included under silage. Australia and New Zealand have large proportions of modified starch corns (50 and 20%, respectively) that are in the total, but *not* further subdivided in Table 14.3.

World total production area (planted) for corn in 1997 was estimated at 140 million hectares. About two thirds was temperate and one third tropical corn. The U.S. grows the most corn with 32 million hectares; about 90% of U.S. grain corn is limited by season. We grow about 10% corn for silage. China is next with 22 million hectares; about 84% of corn in China is late corn, not limited by season. The next rank of corn producers includes Russia with 6 million, the Ukraine and Argentina each with 4 million, then France and Romania each with 3 million, followed by Yugoslavia, Canada, and Hungary each with 1 to 2 million hectares of corn.

The U.S. grows the most early grain corn with 29 million hectares. China and Romania are next at about 3 million hectares each of early grain corn, followed by Yugoslavia, France, and Canada with 1 to 2 million hectares. Russia is the leading early silage corn producer with 4.6 million hectares. The U.S. is next with 3 million hectares. France and Germany each have about 1.5 million hectares of early corn silage.

U.S. seed corn companies give much attention to France and Germany because they use more seed per acre, pay more per unit for more productive seed, engage actively in foreign commerce, and maintain hard currencies. Seed corn business opportunities in France nearly equal the state of Iowa in the U.S.

China grows the most late-temperate, grain corn with 17 million hectares and the most late-temperate silage corn with 1 million hectares. An opportunity appears to exist to increase yield with later, full-season hybrids in China.

I. Goals, Intent, and Mission

When I started breeding corn 40 some years ago, my goal was to develop 50 good, vigorous, S5 inbreds to top cross per year. In a few years, we increased this to 100 good, vigorous, S4 inbreds. We had about 12,000 yield test plots. The station had four full-time people plus seasonal, part-time help. The goal gradually increased to develop a 1000 good, vigorous, S3 families with S4 bulked seed to test cross on two testers per year. We grew to about 25,000 yield test plots. We have developed, by various methods from various materials, several thousand vigorous inbreds good enough to test cross. (I believe quality is more important than quantity.) We still have four full-time people, but *fewer* part-time, seasonal people. Our intent was always to find better ways to do things. How did this increase in efficiency come to pass?

Mechanization has reduced labor. Wire-cone, mechanical, corn planters are a great help. They are flexible for plant densities and for alley widths — which are your two most important needs. You can get any combination of plant density and alley width you want, and plant 600 rows per

TABLE 14.3
Temperate Corn Hectares by Country, by Use, and by Maturity

Country	Total Hectares (000)	Grain Hectares		Silage Hectares		Early/Late (RM)
		Early (000)	Late (000)	Early (000)	Late (000)	
Argentina	3746	30	3615		101	102
Australia	60	13	7	0	10	115
Austria	270	91		179		102
Belgium	110	11		99		102
Bulgaria	350	257		31	62	115
Canada	1170	926		244		115
China	22000	3246	17385	215	1153	102
Czech Rep.	330	150		180		115
France	3200	1270	359	1524	47	102
Germany	1690	276		1414		102
Hungary	1080	954		125		115
Japan	121			30	91	85
Netherlands	80			80		102
New Zealand	25	12		6	2	115
Poland	400	40		360		115
Romania	3000	2636	182	182		115
Russia	6000	1159		4582	259	115
Slovakia	240	109		131		115
Switzerland	55	18		37		102
Ukraine	4000	773		3056	173	102
U.S.	32470	26231	3187	2661	391	115
Yugoslavia	1780	1470	161	60	88	115

Note: Corn acreage estimates by Dr. C. William Crum, Vice President, International Division, DEKALB Genetics, DeKalb, IL 60115.

hour per planter unit. Programmable, gated, seed counters speed seed packaging for multiple locations and for higher plant densities. Corn combines eliminated the increasing harvest cost from increasing yield-test densities. I would prefer two smaller to one larger combine because a greater number of smaller locations will better evaluate hybrids than a smaller number of larger locations. I suppose a double-throated combine would help screen test crosses if several breeders were at one location.

Tom Ishler, who managed foundation seeds for Pennsylvania State University before becoming a commercial corn breeder, recommended isolated plots for making test crosses. He kept records comparing an isolated, detasseled plot with paired rows for making crossed seed. He found that twice as much seed was obtained with about half the time and effort at pollinating time. Female delays are less critical in isolated plots because of multiple planting dates for the male. Isolated plots also provide better plant and ear observations of inbreds than paired rows.

The selection, selfing block is much more efficient. The planter leaves an alley a few cm wide. We hand-plant a hill of sweet corn in every other alley after the machine-planted corn comes up. We now have pairs of ranges back-to-back. We trim a narrow alley (30 cm) between pairs of ranges. This greatly reduces alley effect, and we have more rows on less land with less soil variability. It facilitates selection for higher plant density (HPD) tolerance. We limit number of plants pollinated. We pollinate end plants (see Section IV D), and work alleys instead of working ranges. Data-driven winter-nurseries are a great help in efficiency. Many hybrids are discarded after yield test results have been studied and don't need to be remade.

Personal computers (PC) and label makers have helped. Gene Herrick, assistant manager, had most of one wall in his office covered with index card files. He could answer virtually any question about our breeding materials or yield test entries in a couple of minutes. Your PC can serve this important record keeping function. Use the PC memory as the original source. To my knowledge, we've only mixed up the seed once. I told some people, "It looks like an early MM1, but it is actually an early B37." It was an MM1. Believe your eyes. One digit in a handwritten lot number was misread. PC-generated labels and use of original sources would have kept it straight. Avoid PC software created for plant breeders of self-pollinated crops. You need easy and accurate determination of inbred averages and inbred general combining ability (GCA, the average ability of an inbred to transmit a performance trait to its crosses, see Section IV E). It is your *highest* computing priority.

J. Introduction Summary

This chapter is about commercial corn breeding of temperate corn, which is usually limited by season GDU accumulation. Corn breeding is dynamic; it is important to keep learning about corn and about breeding methods. Try to understand the plant. Early corn grows faster and matures sooner than later corn. Seasons differ. Maturity designations (days RM) are useful to help grow corn where it can properly mature to obtain maximum yields. Most of the corn in the world is limited by season length; China's large amount of late corn is an exception. Effective research stations are well organized. Find better ways of doing things. Learn leadership. Help your boss succeed. Organizations thrive on teamwork. Understand and help accomplish the research plan and the business plan for your company. Satisfy customers by knowing their needs.

II. USEFUL GERMPLASM, PEOPLE AND PLANTS

A. Forward

This section is a shorter, differently focused adaptation of an earlier work.[12] Information on more varieties, more inbreds, and two synthetics is added. Most genealogy is removed. Fewer arguments on the importance of adaptedness are included. Details on temperate and tropical environments are reduced. Details on local environments where germplasm was developed (places) are omitted. The ultimate measure of adaptedness for temperate corn germplasm is the total area on which it was grown. This section lists the more successful varieties, inbreds, and synthetics with their traits, the breeder (if known), and how they were selected. In addition to providing a summary of what has occurred (background of useful germplasm), this section provides proven breeding objectives and proven methods for achieving them. I hope one or more of these examples inspires you to become a better corn breeder.

B. Background

Corn was domesticated 5000 to 8000 years ago in tropical, southern Mexico. The tropics are species rich; temperate areas have fewer, but more widely adapted species.[13] Temperate area environments are more variable and more stressful than tropical area environments. Northern flint and Southern dent are the two races from which U.S. Corn Belt dent corn evolved.[14,15] Flint corn arrived in the U.S. 2500 years before dent corn, which arrived soon after Columbus. Northern flints are earlier and lower yielding; Southern dents are later and higher yielding (Table 14.4). Crosses of dents and flints with subsequent natural and human (artificial) selection provided better adapted, higher yielding varieties for all environmental niches in the U.S. Human (artificial) selection was important. Early selection methods and corn shows emphasized good ears because varieties differed for number of good ears.

Developing open-pollinated varieties required ongoing selection. Successful seed corn companies spanned human generations because of the time required to modify an open-pollinated variety

TABLE 14.4
Some Discriminating Traits between Northern Flints and Southern Dents

Trait	Northern Flint	Southern Dent
Modern breeding	Little	Much
Yield level	Low	High
Flowering date	Earlier	Later
Photoperiod preference	Longer days	Shorter days
Temperature preference	Cooler	Warmer
Physiological balance	Usually source limited	Usually sink limited
Internode number	Fewer	More
Shape	Longer, thinner	Shorter, thicker
Pattern	Progressively longer	Progressively longer to ear attachment node where condensed, then progressively longer
Tassel shape	Smaller, wirier	Larger, thicker
Branch number	Fewer	More
Florets	Sparser	More condensed
Leaf number	Fewer	More
Width	Narrower	Wider
Length	Longer	Shorter
Color	Lighter green	Darker green
Shank	Stout	Slender
Internodes	Longer, thinner	Shorter, fatter
Ear attachment	Larger	Smaller
Internode pattern	Progressively shorter, not condensed	Progressively shorter and condensed at ear attachment
Internode surface	Ribbed	Smooth
Husk number	Fewer	More
Width	Narrower	Wider
Cover	Looser	Tighter, bottleneck
Flag leaves	Usually present	Usually absent
Ear shape	Longer, thinner	Shorter, fatter
Row number	Fewer (8 to 10)	More (14 to 12)
Kernel type	Flintier	More dent-like, gourdseed
Size	Small to large, wide	Large, deep
Shape	Round to flat, wide	Rectangular to flat
Color	Mixed	Usually white
Texture	Harder, corneous starch	Softer starch
Test weight	Higher	Lower
Drying rate	Slower	Faster
Prop roots	Usually absent	Usually present
Tillers	Usually present	Usually absent
Cold germination test	Very good	Average to poor
Seedling growth	Much faster	Slower
Juvenile growth	Faster	Slower
Northern leaf blight	Some tolerance	Susceptible
Southern leaf blight	Susceptible	Some tolerance
Heat tolerance	Susceptible	Some tolerance
Cold tolerance	Some tolerance	Susceptible
Stay-green	Less	More

Source: Brown and Anderson.[22]

and to establish a reputation. Westward and northward movement of agriculture during U.S. westward expansion caused the need for new corn varieties with more drought tolerance and with earlier maturity.[16] Higher-yielding conditions favored the development of some varieties, while stressful conditions favored the development of others due to natural selection. A few varieties were more widely adapted and more popular than others. These widely adapted varieties, though greatly outnumbered (91 to 5) in the initial development of inbreds for hybrid corn, persisted and today make up more than 70% of the background of today's hybrids, because they contained more genes for adaptedness to the U.S. Corn Belt environment.[12,17] Hybrids eliminated the justification for local adaptation. Where the seed was produced no longer mattered.

Cultural practices are ways of modifying environments to help genotypes adapt. Average U.S. Corn Belt plant densities have tripled in 100 years and doubled in the last 40 — drastically changing conditions of life. Selection against silk delay and for good ear development in corn at higher plant densities is survival of the fittest in its purest form. It develops tougher inbreds and hybrids that are more widely adapted.[4,18,19,20] Higher plant densities, more testing sites for evaluation, and modern information management helped develop more widely adapted hybrids.[18,19,20,21]

C. Northern Flint Varieties

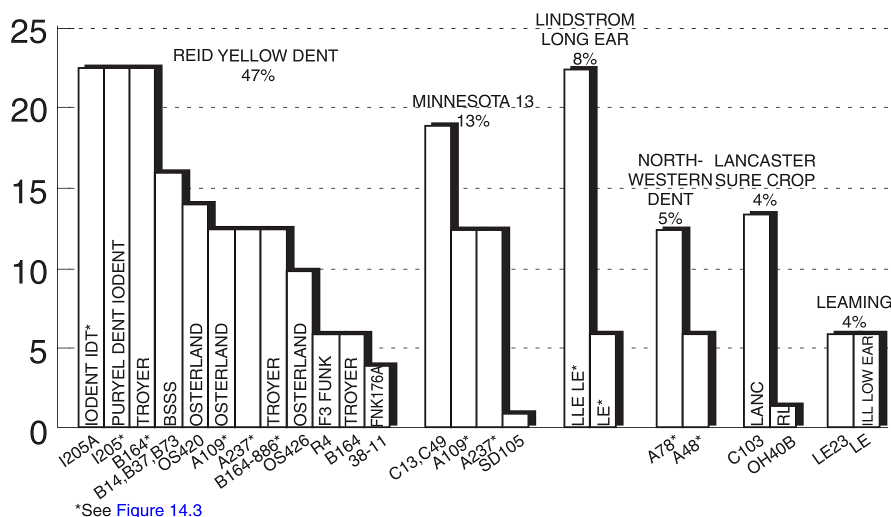
Brown and Anderson described the Northern flints, also known as New England flints and Northeastern flints.[22] They clearly saw Northern flints as being the native, common corn in New England for some time, and they realized flints were being displaced by earlier varieties of dents as they were developed. They stated that flints were widespread in the eastern U.S. in pre-Columbian times and that no other type of corn has been found there in archaeological remains. Flint corn had a 2500-year earlier start in adjusting to shorter seasons, to cooler temperatures, to more variable rainfall, and to longer days.[22]

1. Howe's Alberta Flint (PI 214194)

Alberta Flint was a common corn grown in Alberta, Canada. Ears are 15 to 18 cm (6 to 7 in.) long with 8 to 10 kernel rows on white cobs. Kernel color is yellow. Plant height is 99 cm (40 in.), and ear height is 20 cm (8 in.). It flowers 39 days after emergence. Growth under cool conditions (spring vigor) is excellent. It is very susceptible to common smut, and is extremely early, very short, and it tillers profusely. It often has two and sometimes three ears on the main stem and one ear on some tillers when grown at moderate densities.[23] Alberta yielded poorly *per se*, and also yielded poorly in hybrids with poor GCA. It is listed because it has been a useful source of earliness in the past (Figure 14.2).

2. Longfellow Flint

Longfellow Flint probably originated near Byfield, MA. Byfield is the location of the Longfellow family's original homestead. Mr. Henry Wadsworth Longfellow, poet and academician, left records of Longfellow Flint using the possessive case as if the variety originated with him.[24,25] Galinat states Longfellow Flint seems to be a yellow endosperm form of Rhode Island White Flint.[24,26] Ears are 28 cm (11 in) long, 3.7 cm (1.5 in.) in diameter, and have eight kernel rows on a white cob. Kernels are medium size, medium depth, and yellow to dark yellow in color. Stalks are medium height, medium diameter, and have few tillers. It flowers 70 days after emergence. Mr. Perry Collins developed inbred LF5 (see Figure 14.2). He selected for strong silking at flowering time. He typically used the pedigree method, but his materials were usually BC1s of elite inbreds (see Section IV G). In the late 1950s, LF5 was in the pedigree of virtually all early (105 RM or less) Pioneer hybrids. It contributed earliness, yield, and harder kernel texture.



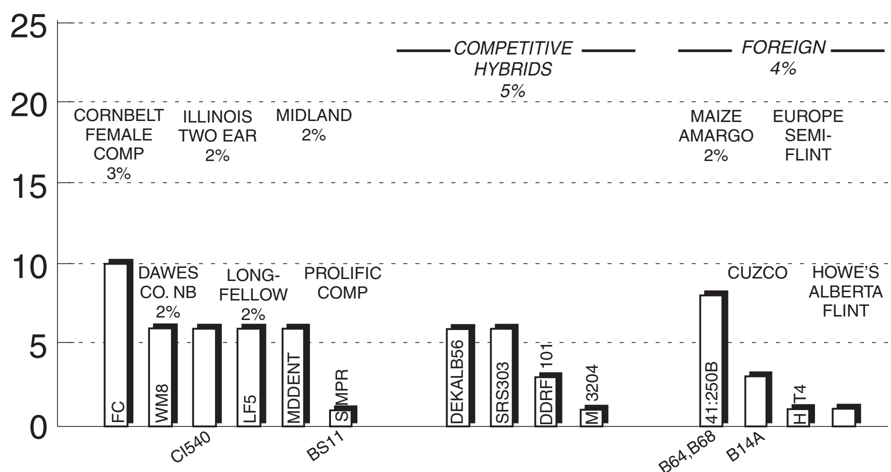


FIGURE 14.2 Background of U.S. hybrid corn. Pedigree background frequencies for 33 Pioneer Hi-Bred Int'l. elite, proprietary inbreds with parental background above and with publicly available representatives below (adapted from Smith et al.[52]).

3. Saskatchewan Flint (PI 214402)

Saskatchewan Flint was a common corn grown in Saskatchewan, Canada. Ears are 12 to 15 cm (5 to 6 in.) long with 8 to 12 kernel rows on white cobs. Kernel color is white. Plant height is 107 cm (42 in.), and ear height is 18 cm (7 in.). It flowers 39 days after emergence. Growth under cool conditions (spring vigor) is excellent. It is susceptible to common rust. It is extremely early, very short, and it tillers profusely. It often has two ears on the main stem and one ear on some tillers when grown at moderate densities.[23] Saskatchewan Flint yielded highest *per se*, but was average yielding in hybrids with average GCA.

4. Motto Flint (PI 245134)

Motto flint was a common corn collected in Russia. Ears are 15 to 18 cm (6 to 7 in.) long with eight kernel rows on white cobs. Kernel color is white. Plant height is 119 cm (47 in.), and ear height is 23 cm (9 in.). It flowers 40 days after emergence. Growth under cool conditions (spring

vigor) is excellent. It is extremely early, very short, and it tillers profusely. It often has two and sometimes three ears on the main stem and one ear on some tillers when grown at moderate densities.[22] Motto yielded average *per se*, but was the highest yielding in hybrids with the highest GCA.

5. Miscellaneous

Cuzco and Maize Amargo in Figure 14.2 were used as donor parents for common rust and for European corn borer resistance, respectively. Cuzco is a very large kerneled flour variety from Peru. Maize Amargo is from Brazil; the name means "bitter" corn in Portuguese.

D. Persistent Open-Pollinated Varieties

Pedigree background frequencies indicate how often particular ancestor-inbreds are represented in the lineage of a group of descendent inbreds. Figure 14.2 represents 33 elite inbreds developed by Pioneer Hi-Bred International. It directly estimates background of >40% of U.S. hybrid corn hectarage and indirectly estimates the rest.[12] It represents their complete product line of more than 100 hybrids for North America because additional inbreds in those commercial hybrids are sister lines to the 33 inbreds represented in the graph. Figure 14.2 is probably representative of the entire industry (percentages will differ by company) because all companies started with the same public inbreds and subsequently selfed superior, competitor's hybrids. I have searched in vain for additional, significant (>1%), background open-pollinated varieties. Krug open-pollinated variety left the scene in the last decade after the demise of inbreds Mo17 and LH51.

An open-pollinated variety was necessarily widely adapted before it was widely grown. Its genes for adaptedness were garnered by natural selection based on climate, on soil type, and on human (artificial) selection based on personal preference. The breeder's heritage, experience, training, and changing times affected variety development.[17]

Stevens has suggested a hypothesis to explain better the species richness of the tropics and the steady decrease in number of species from the equator to the higher latitudes (poles).[13] Stevens attributes this gradient to more environmental variation (varying day length, varying temperature, and varying rainfall) of the higher latitude temperate areas that require wider adaptedness of species. Previous investigators have emphasized the greater number of species in the tropics while Stevens emphasizes the wider adaptedness of the fewer species at higher latitudes. Farmers, seed corn companies, and corn breeders want widely adapted hybrids to better accommodate seasonal variation.[17]

Crosses of dents and flints with subsequent natural and human (artificial) selection provided 1000 better adapted, higher yielding varieties for all environmental niches in the U.S.[16] Some varieties were more widely adapted and more popular than others were. These few, widely adapted varieties were parents of 10% (36 of 367) of the outstanding inbreds in the first decades of hybrid corn.[27] However, their germplasm persisted, then prevailed, and now makes up more than 70% of the background of today's hybrids. The widely adapted varieties contained more genes for adaptedness to longer day length, to lower minimum temperature, to less rainfall, and to shorter season length of the U.S. Corn Belt environment (see Figure 14.2).[17]

1. Funk Yellow Dent Reid and Strain 176A

Funk Yellow Dent Reid was developed from Reid Yellow Dent by Eugene "Gene" D. Funk, Sr. Selection was for the rough, deeply dented show type of ear and for disease resistance. Ears resemble Reid Yellow Dent. Kernels are medium yellow with a light, pale-yellow cap. The kernels are not as compactly placed on the ear as in many strains of Reid Yellow Dent. Some strains of Funk Yellow Dent have been bred for higher content of protein and oil.[28,29] It was recommended by the Delaware Experiment Station in the *1936 USDA Yearbook*. Funk Yellow Dent was the source of inbreds A and

R4, both developed from the same open pollinated ear by Dr. James R. Holbert. In later years Strain 176A, a utility, smoother-eared variety, was developed from Funk Yellow Dent Reid by Dr. Holbert. It was recommended by the Illinois Experiment Station in the *1936 USDA Yearbook.* In 1915 he collected 4200 sound, disease-free ears mostly from disease-free plants, but some were selected from cribs and from seed production plants. He discarded 3000 ears based on germination tests, selecting for ears with freedom from molds and from rotting that possessed unusually good seedling vigor and root development. He then grew 1200 ears ear-to-row in isolation with 100 or more hills per row. He weighed each row and kept the best 20. After additional germination tests, the remnant seed from the best 12 ears became the parents of Strain 176A. A rogue plant in an ear row of Strain 176A was the parent of popular inbred 38–11. Mr. Ralph R. St. John developed it at Purdue University. Strain 176A and Funk Yellow Dent Reid account for about 3% of the background of U.S. hybrid corn tracing back to Indiana inbred 38–11 and Illinois inbred R4, respectively (Figure 14.2).[12]

2. Iodent Reid

Iodent is an abbreviation of Iowa Experiment Station Reid Yellow Dent. Perry Holden and Iowa State College spread Reid Yellow Dent throughout Iowa beginning in 1902. Prof. Lyman C. Burnett started selecting for earliness in Reid Yellow Dent in 1909 using the ear-to-row method and continued at least 13 years. Wallace describes Iodent Reid as an early, rather smooth strain of Reid Yellow Dent with horny, shiny kernels showing very little soft, white starch.[30] Dr. Jenkins selfed Iodent Reid at Iowa State College resulting in inbreds I159, I205, and I224 in the 1920s. Iodent Reid is about 12% of U.S. hybrid corn background (Figure 14.2); virtually all as Modern or Early Iodent inbred derivatives.[12]

Modern Iodent inbreds are the result of a breeding project started in the 1930s by Mr. Raymond F. Baker at Johnston, Iowa (Figure 14.3).[31] Inbreds Idt and I205 were separated during inbreeding (probably at S3) at Iowa State by Dr. Jenkins, who finished I205, and Idt was finished by Dr. Ernest W. Lindstrom. Baker used Idt × I205 as the male of Hi-Bred 330. Inbred Idt had good stalks and roots but sort of “just went along” for yield. Baker used B164 by LE (long ear) as the non-recurrent source of high yield with Idt as the recurrent backcross parent. He grew 16 ha (40 acres) of breeding populations as a detasseled portion of a production field with Idt × I205 as the male resulting in seed that was 87.5% Iodent. He selected for ear length at harvest, using ears of the male as comparative checks. He kept fewer than 200 ears from the B164 × LE non-recurrent portion of the field. These were grown ear-to-row for two generations and selected for longer ears and for stronger germination before test crossing. He had planned to use the related cross A × R4 (female of Hi-Bred 330) as tester, but newer materials were substituted. These cycle 2 selections resulted in three very important commercial inbreds.

Early Iodent inbreds resulted from the third cycle started at Mankato, Minnesota in 1958 (Figure 14.3). I was fresh from the University of Minnesota where I worked with Dr. Ernest H. Rinke. I was enthusiastic about his method to make popular inbreds earlier (see E.6.). I discussed the method at length with Mr. Baker.[32] Our objective was to develop earlier versions of his late Modern Iodent inbreds. He suggested using two of the faster drying Modern Iodent inbreds for late (adapted in southern and central Iowa) recurrent parents. He chose one early inbred with Iodent in its background (25%), and I chose an early single cross with B164 in its background as early (adapted in south central Minnesota) donor parents. Each of the three early inbreds contributed excellent yield and stalk strength to hybrids. I did the selection at Mankato, MN and at Homestead, FL. Segregating populations were grown at 87,500 plants per hectare (35,000 per acre) — more than twice normal for the period. I typically grew 1000 to 1200 plants of each segregating population and chose the earliest flowering 10 or 12 plants (1%) to self, to backcross, or to sib mate. At harvest the best ears from the best plants were selected.

Dr. Rinke presented a paper on “Moving the Corn Belt northward” at the ASTA meeting in Chicago in December, 1961.[32] Mr. Walter E. Vandeventer of Tipton, IN listened attentively to the

1920s 1940s 1950s 1960s 1970s (early) 1970s (late) 1980s

Idt[2] (B164 x LE)
Idt x I205
C49 x Idt
C49
Idt[2] (B164 x LE)
Idt x I205
B164-886
A237
A78
A109

Idt)2B
C49)2A
Idt)2C
A556
A509

Idt)3A
Idt)3C
Idt)3B

Idt)4B
Idt)4A

Idt)5A
Idt)5B

Idt)6A

1st cycle 2nd cycle 3rd cycle 4th cycle 5th cycle 6th cycle

FIGURE 14.3 History of Iodent germplasm development. Example of cumulative selection (recurrent selection with late testing) practiced over 60 years. See Figure 14.2 and text for background of these inbreds. (Courtesy of Raymond Baker (deceased), adapted from Hallauer and Miranda.[50])

paper, then asked Mr. Baker and me for more information on the method. He later asked me for seed of 20 families totaling some 5000 seeds that had been selected for early flowering, to self or to backcross, for five generations. He grew 111 rows of these materials in 1962 and pollinated the earlier plants of each pedigree as well as some later-than-average plants. A total of 41 ears (approximately 1%) were saved. The first top crosses were grown in 1965 and 1966.

In 1966, Dr. L. John Hoffbeck joined the Tipton, IN station to enlarge the early corn breeding effort. From the above materials three useful inbreds were developed. [In Figure 14.3 under 1950s, early sources are C49)2A (25% Iodent) and A509 × A556; late inbreds are Idt)2B and Idt)2C (each 88% Iodent).] Idt)3A was Early × Late selfed and selected for earliness and then backcrossed to Late (72% Iodent); Idt)3A was very important commercially in a popular single cross and in several three-way-cross hybrids. Idt)3B was Early × Late) × Late selfed and selected for earliness, and then backcrossed to Late again (80% Iodent). Idt)3B was derived from one of the 1962 later flowering plants. Idt)3C was Early × Late selfed and selected for earliness and then backcrossed two times to Late (80% Iodent).

A fourth cycle of selection was started immediately. About 800 plants were grown in each S1 (F2) at 100,000 plants per hectare (40,000 per acre). The earliest 80 plants (10%) were selfed and the 8 to 10 (1%) best ears from the best plants were saved at harvest and advanced at Homestead, FL. About 40 S3 (F4) ears per family were grown ear-to-row at 100,000 plants per hectare (40,000 per acre) at Tipton and the best 2 (5%) rows saved based on phenotypic selection. Test crosses were then made on a single-cross tester and grown in northern Indiana in 1971 and 1972. Dr. Hoffbeck developed Idt)4A (76% Iodent), which was extremely important commercially. It was probably grown in hybrids on more hectares than Minnesota inbred A632 and French inbred F2 together.

The fifth cycle started at Mankato, MN in 1973. My objective was to increase husk length of Idt)4A from Idt)4B. About 1000 BC1 plants were grown at 75,000 plants per hectare (30,000 per acre) in our leaf-disease nursery and the earliest 100 (10%) plants selfed and 19 ears saved from the best plants based on phenotypic selection for strong silking, for stay-green, for leaf-disease tolerance, and for ear development. These were advanced at Homestead, FL, with selection for juvenile vigor, for leaf-disease tolerance, and for well-developed ears. The resulting 42 ears from 19 families, S3 (BC1S2) ears, were selfed at Mankato, and they were observed at Algona, IA, and at our lake bottom, leaf-disease nursery. The rows were evaluated for six agronomic traits plus disease and insect tolerance. Most emphasis was on ear development and on stay-green. Ears were saved from 11 S3 rows, with selection on a row basis. The two best rows were crossed to inbred testers at Homestead, FL. The test crosses were grown in southern Minnesota and in our leaf disease nursery in 1977 and 1978. Dr. Robert W. Rosenbrook increased these inbreds, crossed them onto more inbreds, and ushered their newer hybrids through the advancement process. Idt)5A and 5B (each 76% Iodent) were very important commercially; Idt)5A was a parent of a popular commercial hybrid.

The sixth cycle started at Algona, IA in 1980. Mr. William B. Ambrose grew the S1 (F2) population of Idt)5A × Idt)5B near Algona. He grew 600 plants at 70,000 plants per hectare (28,000 per acre) and saved seven ears. He sent these ears to Dr. Thomas J. Kevern, who grew the S2, S3, and S4 generations at 70,000 plants per hectare (28,000 per acre) near Janesville, WI and selected two, one, and three ears, respectively. Dr. Kevern selected for synchronous flowering and for anthracnose stalk rot resistance. He made test crosses to two inbred testers and evaluated them in the northern U.S. Corn Belt. Idt)6A (76% Iodent) became a parent of a popular commercial hybrid.

Figures 14.2 and 14.3 represent a 1990 snapshot of successful inbred development with a 70-year depth of field. Raymond Baker was the guiding force. Darwin would call Figure 14.3 breeding in and in, and animal breeders would call it line breeding or close breeding because so much backcrossing and so much use of related materials occurred. I call it genome conservation.

3. Lancaster Sure Crop

Lancaster Sure Crop variety was recommended by state experiment stations in Connecticut, Delaware, New Jersey, Pennsylvania, and West Virginia in 1936[27]; it was popular in the eastern U.S. Over time Lancaster Sure Crop was out crossed to later dent cultivars then crossed back to Lancaster Sure Crop 8 to 12 times by mixing seed in the field, followed by selection for flintier, smoother, longer ears. Uniformity was lacking for the first 50 years because of outcrossing to dent varieties. Its maturity is medium early (112 RM) for the central U.S. Corn Belt. Ears are 25 to 30 cm (10 to 12 in.) long, slender, smooth, and have 10 to 14 kernel rows, white cobs, and large diameter shanks. Kernels are light to medium yellow, large, deep, rounded, and flinty. Plants are tall with medium high ear height and only average root and stalk strength.[33,34] Lancaster Sure Crop variety accounts for about 3% of U.S. hybrid corn background (Figure 14.2). Virtually all of it traces back to inbred C103.[12]

Jacob Hershey came to depend on an early, slender, smooth, usually single-eared corn variety. Henry High in nearby Byerston originally obtained it from the U.S. Patent Office in 1860. The common corn grown locally was a large, late corn with medium to rough dent. Jacob mixed seed from two or three selected ears of this common corn with his pile of shelled seed corn. This was repeated a number of times and later Jacob's son John and John's son Jacob repeated the process six or eight more times with various dent varieties. One year a poor stand of Golden Queen was replanted with Lancaster Sure Crop and some resulting cross-pollinated seed saved.

The mixing of varieties stopped in 1910. Their main interest was in high yielding corn for their own use, but they eventually got into the seed corn business. Isaac, helped by his brother Benjamin, selected among harvested ears in the seed house. He preferred medium-length, well-matured, sound ears with clean shanks, and with neither moldy nor silk-cut kernels. He repeatedly selected smoother, flintier ears. The corn was called Sure Crop because of earlier-than-average maturity and drought

tolerance. Isaac's son, Noah, later selected for a larger ear and a better root system by selecting ears from standing corn in the field.[12]

4. Leaming Corn

Leaming Corn was the first popular corn variety.[35] It was grown in all parts of the U.S. and increased in popularity near the end of the 19th century to become grown more than all other yellow, open-pollinated varieties when about half the corn grown was white varieties. Arkansas, Delaware, Kentucky, Nebraska, Ohio, Pennsylvania, and West Virginia experiment stations recommended it in 1936.[27]

Leaming Corn is described as medium late maturity (120 RM) for the central U.S. Corn Belt. Ears are about 23 cm (9 in.) long, 18 to 19 cm (7 to 7.5 in.) in circumference, and markedly taper. They have moderately rounded and expanded butts, reduced number of kernel rows in the middle and toward the tip of the ear, and 16 to 24 kernel rows in distinct pairs. Butts and tips are usually well filled. Cobs are medium red color with medium to large shank attachment. Kernels are long, rather narrow, thick, loose, wedge shaped, and have nearly straight edges with dimpled to pinched dent. Tip kernels are pointed. Kernels are colored yellow-orange. Stalks are 3 m (10 ft) tall with medium-low ear attachment. Leaming Corn gained international prominence by winning gold medals in Europe.[29,36,37]

Dr. Edward M. East's classical inbreeding and hybridizing experiments at Illinois and Connecticut Experiment Stations used Leaming Corn and Burr White inbreds. Dr. Donald F. Jones' first double-cross hybrid used these same Leaming inbreds. Dr. Louie H. Smith used Leaming Corn in divergent ear height selection experiments at Illinois. Henry Wallace's 'Copper Cross', one of the first hybrids sold in Iowa (1924), had one of these Leaming Corn inbreds as female parent with an inbred from Bloody Butcher as male parent. Inbred LE23 (a parent of Stiff Stalk Synthetic) is from Leaming Corn via Illinois Low Ear. Inbred L is from Leaming Corn and crossed with CI540 from Illinois Two Ear resulted in inbred Oh07. Leaming Corn is about 4% of the background of U.S. hybrid corn tracing back to Ohio inbred Oh07 derivatives (Figure 14.2).[12]

Christopher Leaming moved from New Jersey and settled near Cincinnati, OH in the early 1800s. Virtually all corn was grown in the river bottom fields where Miami Indians had grown it. It was customary for hill-land farmers to sharecrop (2/3 tenant, 1/3 owner) bottom land for corn growing. It was very labor intensive. Corn yields averaged about 31.3 q/ha (50 bu/acre) in 1827 when Christopher raised a 4 ha (10 acre) plot of corn averaging 65.2 q/ha (104 bu/acre). It was grown on the Langdon Bottoms floodplain, south of Red Bank Station on the west bank of the Little Miami river (Section 14, Spencer Township, Hamilton County, OH). As you cross the bridge on OH 125, the field is upstream to the left (northwest). He attributed the high yield to elimination of weeds and to deeper plowing. Christopher selected the corn for planting from the Common Yellow Indian corn indigenous to the lower Miami River Valley area. His extraordinary corn yield was an inspiration to corn growers everywhere. He was the father of Jacob S. Leaming, born in 1815.[35]

Jake Leaming was born and raised near Madisonville and the Little Miami River just east of Cincinnati. He attended district schools and taught school for a time, operated a coach line, and then went back to farming. Jake's eighth child Peter, born in 1856, gives the following prenatal account of the origin of Leaming corn: In the autumn of 1855, Jake was out for a drive along Bullskin Run near Cincinnati and stopped at a farm to feed and to rest his horse. He was so impressed with the soundness of the ears and their deep yellow color that he bought a bushel for seed corn. He moved the next spring to Wilmington, OH (56 km northeast) where he grew and selected the corn for 30 years. Peter's account was given in 1911. Lloyd argues that Jake's corn was not directly descended from his father's corn.[35]

Jake's selection included removing weak and barren plants before pollination, and then marking early ripening ears (with twine) on two-eared plants. These ears were more often tapered and, over time, selection resulted in a noticeably tapered ear due to reduced number of kernel rows. He was

innovative; he was an early user of legumes in his crop rotation (corn, wheat or oats, and red clover), and he experimented with drilled spacing. He was elected trustee of Union Township, Clinton County, OH. Jake and his wife Lydia Ann were active members of the Methodist Church; they had seven sons (and two daughters) charged with keeping the corn weed-free. Jake grew several 62.7 q/ha (100 bu/acre) yields in favorable seasons, and for this reason, became well known as a corn authority. He shipped seed corn to all parts of the U.S. He was the first nominee to the Ohio Farmers' Hall of Fame.[35]

5. Minnesota 13

Minnesota 13 was the most popular variety both in the northern U.S. Corn Belt and also at higher elevations; it was recommended by the Arizona, Colorado, Idaho, Minnesota, Montana, Nebraska, Nevada, New Hampshire, North Dakota, Oregon, South Dakota, Utah, Vermont, Wisconsin, and Wyoming Experiment Stations in 1936.[27] Less than 400 thousand ha (1 million acres) of corn were grown in Minnesota in 1893 when Prof. Willet M. Hays began work on Minnesota 13 variety; acreage grew steadily to 2 million ha in 1932. Minnesota 13 moved ear corn growing northward 50 miles in the U.S. in a decade (probably 1895–1904).[38]

Minnesota 13 variety is a mid-early (95RM) yellow dent. It is early maturing but heavy yielding, adapted from southern Minnesota northward. Ears are 15 to 21 cm (6 to 8.5 in.) long, 16.5 cm (6.5 in.) in circumference, slightly tapered, and have 12 to 16 kernel rows, medium size red cobs, and medium size shank diameter and length. Kernels are medium yellow, medium deep, and medium broad with a medium smooth, dimpled dent. Kernels are compactly set on the cob. The butts are well rounded and the tips well filled. Plants are 2 to 2.1 m (6.5 to 7 ft) tall, and ear height is 71 to 79 cm (28 to 31 in.) high.[28,29]

Minnesota 13 inbreds were developed at Minnesota (C11, C14, C46, and C49), Colorado, Montana (Mt42), North Dakota (ND203), and Wisconsin (M13, W117, W153R, and 30 some others). The first (1933) popular, early-maturity hybrid in northern Iowa and southern Minnesota was Minnhybrid 301/Pioneer 355 (C11 × C14) B164. Dr. Herbert K. Hayes developed inbreds C11 and C14 from Minnesota 13 at the University of Minnesota. Inbred W117 was very popular in the 1970's. W153R is a parent of Holden LH82, and W22 is a recurrent parent of many genetic stocks. Inbreds Mt42 and ND203 are non-recurrent parents in A632, A634 and in A635, respectively. Important Minnesota 13 inbreds in the background of present hybrids include C13, C49, A109, A237, and SD105. Wisconsin inbred M13 was a grandparent of inbred Oh43. Minnesota 13 variety accounts for about 13% of U.S. hybrid corn background tracing back to Minnesota inbreds A109, A237, C13, C49, and South Dakota inbred SD105 (Figures 14.2 and 14.3).[12,28,29]

Prof. Willet M. Hays was born and raised in Eldora, IA. He attended Oskaloosa, Drake, and finally Iowa State Colleges where he received a B.S. in 1885 and M.S. in 1886. He joined the University of Minnesota faculty in 1888. He helped organize North Dakota State College and Experiment Station in 1891–1892, then returned to Minnesota as professor in 1893.

On April 1, 1893, Mr. Andrew Boss, at the request of Prof. Willet M. Hays, purchased a yellow dent corn variety from DeCou & Co. (North Star Seed Co.) near Kellogg and Robert intersection in St. Paul. Franklin DeCou was proprietor. In the 1894 nursery book the word "common" has been added above the variety name St. Paul and the firm name "DeCou & Co." has been added in the description column. The writing of the additions is the same as the known writing of Andrew Boss. He considered the variety to be the common corn grown in the St. Paul area. A few pounds of this seed were sent out as North Star — almost certainly DeCou & Co.'s name for what became Minnesota 13. Prof. Hays selected the variety by the centgener method for three years. Then 300 available bushels were listed as University No. 13 in 1896; selection and distribution continued through 1910. More than a thousand bushels were distributed as Minnesota 13 at a dollar per bushel to cover production costs.[39]

Prof. Willet M. Hays originated the centgener method and used it on wheat, corn, flax, and dry beans. Centgener method means to grow 100 plants per generation from a single selected plant then select the best plant and repeat. It emphasizes individual plant selection — single plant descent. Later he saved five ears per centgener resulting in the first grid system or modified mass selection. Up to 40 centgeners were grown per variety selected. Corn centgeners were thinned to 31,000 plants per hectare (12,400 per acre) — relatively high for the period. Selection was for more mature ears, for higher yield of dry shelled corn, and for more nitrogen in the grain.[39]

6. Northwestern Dent

Northwestern Dent was the most popular variety in the northwest.[40] Nine northwestern experiment stations recommended it in 1936.[27] Northwestern Dent variety is mid-early to early (80 RM). It is distinctly earlier than Minnesota 13. Ears are 15 to 20 cm (6 to 8 in.) long, slightly tapered, and have 10 to 16 kernel rows on white cobs. Kernels are semi-dent, red in color (pericarp) with white or yellow indentations. Plants are leafy, 1.4 to 1.8 m (4.5 to 6 ft) tall, often tillered and have an ear height of 51 cm (20 in.).[41] Minnesota and Wisconsin Universities developed useful Northwestern Dent inbreds. Most of the Northwestern Dent germplasm in today's hybrids trace through A48 (a.k.a. 64) as Oh43 derivatives, through A78 as A509 derivatives, and through A509 as one source of earliness in Early Iodent inbreds. Northwestern Dent is about 5% of U.S. hybrid corn background (Figure 14.2).[12]

Northwestern Dent was developed and introduced by Oscar Will. Bloody Butcher variety was brought to Bismarck by Mr. Joe W. Burch, who drove a team of horses to Dakota Territory (47th parallel) from 16 km (10 mi) north of Bloomington, IN. (39th parallel) in 1891. Oscar selected 15 or 20 more developed ears from earlier plants before frost killed the rest of Burch's first crop. Oscar repeated selection for earlier plants with more mature ears the next two years. Burch then raised a considerable crop of seed. Oscar first sold Northwestern Dent in 1896, and it quickly became very popular. Northwestern Dent was maintained and selected on the Will Seed Co. farm.[40,41]

7. Osterland Reid

Henry Osterland developed Osterland Reid near Ackley, IA. It was earlier with lower plant and ear height than Reid Yellow Dent. Osterland Reid was recommended by the Iowa Experiment Station in the *1936 USDA Yearbook.* Osterland Reid is the parent variety of inbreds OS420 and OS426, developed by Dr. Merle T. Jenkins at Iowa State University. Famous Iowa Hybrid 939 had OS420 × OS426 as the male. Osterland Reid is also the parent of inbred A26, developed by Dr. Herbert K. Hayes at the University of Minnesota. Inbreds A26 and C49 (from Minnesota 13) were subsequently crossed to develop the Minnesota inbred A109. A number of very early to medium late Osterland Reid inbreds from OS420 × OS426 have existed. Osterland Reid is about 11% of U.S. hybrid corn background (Figure 14.2).[12]

Henry Osterland showed Four County White (improved Silver King) as well as Osterland Reid Yellow Dent. He entered both varieties in the Iowa Corn Yield Test beginning in 1920 and usually finished in the top 20%; Osterland Reid had the highest 10-year average in 1929. He noticed that ears with row numbers divisible by 4 (16, 20, 24) tended to be straight-rowed while others (18,22) tended to spiral. He showed his friend, Prof. Harold D. Hughes, who arranged demonstrations of the phenomenon at the Iowa State field days and at the corn show.

Henry grew seed from ears selected on standing plants with some bare cob at the tip to have room for more kernels in better years. He selected ears that extended beyond the husks to help the husks open up and hasten drying. He liked rather long ears with 18 or 20 kernel rows that were not too large in circumference, heavy with close set kernels of medium dimensions, medium to smooth indentation, and mostly free of soft starch. Henry twice refused the Iowa Master Farmer

Award even after letters of encouragement from H. A. Wallace. Twenty years later he answered a query from Dr. William L. Brown to the effect that he doubted his experiences would be useful to modern breeders. Henry Osterland was humble. He passed away in his 78th year in 1958; neither he nor his two brothers left a son to carry on the seed business.[33]

8. Reid Yellow Dent

Reid Yellow Dent was the most popular variety in the United States; 21 central states recommended it in the *1936 USDA Yearbook.* It was described as medium early relative maturity (115 RM) in the central Corn Belt. Ears are 23 to 25 cm (9 to 10 in.) long, slightly tapered, well filled at the tips and butts, and have 16 to 22 closely spaced, dove-tailed, kernel rows on small, dark-red cobs with small shank attachment. Kernels are medium depth, slightly keystone in shape, medium narrow width, square crowned, and smooth to rough indentation. Kernel color is bright, deep yellow with red tinges and a lighter yellow cap. Stalks are rather heavy, tall, and leafy with above average ear height.[28,37]

Reid Yellow Dent became very popular after winning the World's Fair (Columbian Exposition) corn show in Chicago in 1893. It won countless state and local corn shows throughout the U.S. Corn Belt.[42,43] The corn shows were instrumental in its fast spread.[43] Reid Yellow Dent underwent 50 years of evolution as the dominant variety (estimates up to 75% of yellow corn) in the U.S. Corn Belt.[44] Several modified Reid strains important to present-day U.S. Corn Belt corn evolved (Figure 14.2).

Reid Yellow Dent was the source of inbred WF9 (Wilson Farm row 9). Mr. Benjamin H. Duddleston developed WF9 at Purdue University. Inbred WF9 was probably grown in hybrids on 30% or more of U.S. corn acreage for 30 years, making it the most popular inbred of all time. The merits of WF9 became known after Dr. James R. Holbert made the cross and tested hybrid WF9 × R4 in 1930. Mr. Ralph St. John obtained Dr. Arthur M. Brunson's inbred HY from Dr. Holbert and made the cross and tested hybrid WF9 × HY. Fifty percent of U.S. hybrid corn background is Reid Yellow Dent, including its prominent, persistent strains of Funk, Osterland, Troyer, and Iodent varieties and Iowa Stiff Stalk Synthetic. Reid Yellow Dent *per se* is about 4%; it persists through Minnesota inbred A237 in Early Iodent inbreds (Figures 14.2 and 14.3).[12]

Robert Reid moved his family from Russellville, Ohio (56 km southeast of Cincinnati) to the Delavan plains in Illinois (40 km south of Peoria) late in the spring of 1846. He brought along some Gordon Hopkins seed corn, a semi-gourd seed dent, which he planted in very late spring. He harvested an immature crop and saved the better ears for seed. He failed to check germination, and in 1847 they produced a poor stand that was too good to destroy. He replanted missing hills with Little Yellow, an early native Indian flint corn. His replanting allowed the different maturity corns to cross-pollinate. He used this cross-pollinated seed for planting. He also did the maturity selection that was necessary because one parent was too early and the other too late for productive use in north central Illinois. Robert was an exacting father who pursued excellence as his guiding principle.[43]

James L. Reid was born in 1844 in the Red Oak settlement near Russellville, OH. He came to Illinois as a toddler in the same covered wagon as the gourd seed corn. The oldest of three children, he received the brunt of his father's philosophy. Robert taught James to read at the age of four, to harness a team and to plow a straight furrow at the age of nine, and to practice the rudiments of corn selection soon after. James attended Tazewell College for 2 years and taught school a term before farming on his own in 1867. He was a quiet, thoughtful, ambitious person and a lover of books and of nature, according to his daughter, Olive. He was also described as withdrawn and deliberate by some of his envious competitors. He was a talented artist expressing himself through beautiful ears of corn.

His emphasis on ear selection was for medium size of medium maturity, for bright yellow kernel color with solid deep, relatively smooth grain and a small red cob, and for 18 to 22 kernel

rows being well filled over the tip and butt — leaving a small shank to ease hand husking. He saw vigorous plant growth and high shelling percentage as essential traits. He selected seed in the field at harvest time emphasizing mature, dry seed. Both Robert and James gave seed to their neighbors to keep Reid Yellow Dent pure.[44]

His seed business was modest because of stringent selection. He sold only 5% of the ears from his highest yielding field — providing only 9,545 to 12,727 kg (300 to 400 bu) of seed per year. He was introduced at the National Corn Exposition at Omaha in 1908 by Mr. J. W. Jones, exposition manager, as the man who has put more millions of dollars into the pockets of the farmers in the U.S. Corn Belt than any other man. He was also described by *Country Gentleman Magazine* as the man who put the Corn Belt on the map of America.[45]

9. Richey Lancaster

Richard Crabb in *The Hybrid Corn-Makers* states that David Richey had grown Lancaster Sure Crop corn near LaSalle, Illinois many years before 1902 and that his son, Frank, was still growing it there in 1922. So, Lancaster Sure Crop had natural and human selection in northern Illinois certainly more than 20 years, perhaps several decades more, by the Richey family. It was probably brought to Illinois by migration of Mennonites from Pennsylvania. One of the earliest groups (six families of 45 people) came to northern Illinois (Sterling) in 1867. More came later. They would certainly have brought seed for crops.

Richey Lancaster is the parent variety of inbreds 6–5, CI4-8, OH40B, L3, L9, L289, L304A, L317, and LDG. Dr. Frederick D. Richey (FD) developed inbreds 6–5, CI4-8 and L9 at Arlington Farm, Virginia where the Pentagon now stands. Inbred L9 was a parent of Hoosier Hybrid (see II.E.). Dr. Jenkins developed Iowa inbreds L289, L304A, and L317. Inbreds L289 and L317 were each one of the parents of hybrids IA939 and U.S.13, respectively. All of these inbreds trace back to Richey Lancaster variety collected from the Frank Richey farm by FD. He selfed it himself at Arlington Farm, Virginia and also gave 50 ears to Dr. Jenkins at Iowa State College. FD encouraged Glenn H. Stringfield to make a double-double (eight inbreds) synthetic of Richey Lancaster inbreds from which inbred Oh40B was selected. Stringfield then developed inbred OH43 from hybrid OH40B × W8 (1/2 Richey Lancaster, 1/4 Minnesota 13, and 1/4 Northwestern Dent). Richey Lancaster variety accounts for about 1% of the background of U.S. hybrid corn tracing back to Ohio inbred OH43 and its derivatives.[12]

David Richey purchased 66 ha (164 acres) in Section 10 of Eden township in LaSalle County just south of his father's farm near LaSalle, IL in 1850. David's son Frank became an attorney and practiced in St. Louis where FD was born and raised. Frank later returned to the home farm after his father's death. FD spent his summers on Grandfather David's farm. In the fall of 1903, he helped his ill grandfather on the farm. FD was charged with selecting the longer, heavier, smoother ears for 50 bushels of Richey Lancaster seed corn. At his own initiative, he ran germination tests in cigar boxes and found their more-flinty seed corn germinated better than softer, rougher-eared, more-dented, competitive varieties.

FD Richey enrolled in general agriculture at the University of Missouri and graduated in 1909 with the intent of farming his father's farm. Instead, he took a job with Mr. C. P. Hartley at the USDA Bureau of Plant Industry in 1911. In 1916, he began inbreeding corn to develop inbreds. In 1920 he gave Bloody Butcher inbred material to H. A. Wallace, who selfed it again and used it as the male of Copper Cross hybrid seed corn sold in Iowa in 1924. In 1922, FD became principal agronomist in charge of USDA corn improvement; he took 50 ears of Richey Lancaster corn from his father's farm near LaSalle, Illinois to Dr. Jenkins at Iowa State College for inbred development.[43]

FD was Chief of the Bureau of Plant Industry in USDA from 1934 to 1938. He received the Distinguished Service Award for outstanding service in organizing and leading the cooperative corn-breeding program, which gave hybrid corn to American agriculture. He received an honorary

Doctor of Science degree from the University of Missouri in 1949. He was the author of numerous publications on corn growing, corn breeding, and statistical methods including a paper on cumulative selection that accurately predicted future corn breeding methodology.[46] FD Richey retired after 37 years of USDA service and 7 years of private or state service.[47]

10. Troyer Reid

Troyer Reid was popular at corn shows. The Indiana Experiment Station recommended it in the *1936 USDA Yearbook.* It was described as adapted to northern Illinois, Indiana, and Ohio (110 RM). Ears are large, cylindrical shaped, 23 to 28 cm (9 to 11 in.) long, 18 to 20 cm (7 to 8 in.) circumference, 429 to 471 g (15 to 20 oz) when thoroughly dry on small diameter red cobs. Exceptionally well-filled butts make it easy to husk. Kernels are large, deep, broad, and uniform sized. Kernel color is bright, shiny, medium yellow. Stalks are strong with medium ear height. Troyer Reid has larger and more uniform ears, broader and deeper grain, and slightly rougher indentation than Reid Yellow Dent.[48]

Troyer Reid is the parent variety of three public inbreds (461-3, B164, and Tr) and probably two more (B2 and Fe). Inbred B2 is named for Butler farm near Indianapolis. Mr. Butler bought seed from Chester E. Troyer. Inbred Fe tolerates iron uptake, a trait also present in 461-3 (Troyer 9-1-1) and Tr (461–5, Troyer 9-1-1–6). The evidence for B2 and Fe being derived from Troyer Reid is hearsay. Inbreds 461-3, B2, Fe, and Tr are 4 of 18 parental inbreds in Stiff Stalk Synthetic.[49,50] Duddleston developed these inbreds at Purdue. Besides its contribution through Stiff Stalk Synthetic, Troyer Reid is in Modern Iodent and Early Iodent inbreds through inbreds B164 and A556. About 12% of U.S. hybrid corn background is Troyer Reid (Figure 14.2) as Modern and Early Iodent inbred derivatives.

In 1924 Mr. Henry A. Wallace (corn breeder, entrepreneur, and government official) visited Purdue.[33,51] He saw Duddleston's 461 (Troyer 9-1) growing vigorously on a run-down farm and on artificial soils low in phosphate and potash in the greenhouse. Wallace left with bulked S2 or S3 seeds of 461 from Dr. Hoffer. He increased it and gave it to Mr. Raymond F. Baker to make experimental hybrids in 1926.

Baker was an Iowa State undergraduate at the state corn show when his instructor, Professor Edward R. Henson introduced him as a very good student to Wallace. The following summer, Baker, with his mother's help, grew a 461-male isolation on the home farm near Beaconsfield, Iowa and detasseled the females. Wallace's interest in 461 waned, but Baker persevered to enter 'Hi-Bred B1' in the 1927 Iowa Yield Test where he won the Banner Trophy for the highest yielding entry in the state trials. Wallace then hired Baker to do corn breeding for the Hi-Bred (now Pioneer Hi-Bred International, Inc.) Corn Co. in 1928. After further selfing and selection by Baker within 461, inbred B164 (B for Baker, 164 for 461 reversed) was developed.

Wallace traded inbred B164 to Dr. Herbert K. Hayes of the Minnesota Experiment Station for inbreds C11 and C14 in 1930.[27] Inbred B164 was the male parent of Hi-Bred 355 and of Minnhybrid 301 — the first (1933) popular hybrid in southern Minnesota and northern Iowa. The female was single cross C11 × C14 from Minnesota 13 variety. Inbred B164 was a parent of Modern Iodent inbreds (see II E 5, Figure 14.3).[30,52] Inbred Tr was a parent of Hoosier Hybrid, the first (1935) commercially available hybrid in Indiana. The other two parents were inbreds 66 from Early Yellow Dent and L9 from Richey Lancaster. Mr. Ralph St. John developed the hybrid. In 1942, inbred Tr was the fourth most popular inbred (after WF9, 38-11, and Hy, but before L317) in the five central Corn Belt States.[53]

David Troyer, my grandfather, obtained Reid Yellow Dent between 1894 and 1900. He carefully removed husks, silks, and other nesting materials from ear corn before cribbing to reduce rodent damage. In fact, the clean ears dried better in the fall, and seed from the drier ears grew better the following spring. Neighbors noticed his good stands of corn, and asked to buy seed corn. My Uncle

Chester, Dave's older son, used a Barlow knife to examine germs on kernels from each seed ear to be sure it hadn't frozen.[54]

Chester E. "Chet" Troyer attended nearby Marion Normal College taking scientific and education courses. He taught in a district school 2 years and was the principal of La Fontaine High School 3 years. Chet married his best pupil (Cleo Hamilton) in 1908 and concentrated on the seed corn business. He placed high (6th in 50,000) in the National Corn Exposition in Omaha in 1909.[54] First prize was a section (256 ha) of farmland. Chet was the consummate corn showman; he was competitive. He was four times Corn King of the World, winning the grand champion ten-ear sample four times at the International Grain and Livestock Show in Chicago. His closest competitor was friend Peter J. Lux of Shelbyville, Indiana who won twice. Chet won repeatedly against tremendous odds. He selected beautiful ears of corn. His former home is a memorial library in La Fontaine. Troyer Bros. catalogs describe improving Illinois Reid by breeding, by selection, and by introduction of a few choice ears from the most promising sources (other Reids?).[48,54]

E. Popular Inbreds

Elite inbreds in the U.S. Corn Belt are at the pinnacle of evolution for their area because they have a longer history of development; many generations of selection have occurred and more intense competition among breeders has occurred where more resources, both people and money, have been expended. Elite inbreds are the best source materials for future progress.[55]

1. 38-11

Mr. Ralph R. St. John developed 38-11 from a rogue plant in a selfed ear row in Strain 176A at Purdue University. He inherited Duddleston's yellow corn inbred development program when the latter went on sabbatical. It was released in 1936. WF9 × 38-11 was the female of U.S.13 and of many, many other double crosses. Inbred 38-11 was the second or third most popular inbred, alternating with HY, for more than 20 years.[53] When not used with WF9, 38-11 was usually in later hybrids for the southern part of the central Corn Belt. It contributes vigorous growth, extensive root systems, leaf blight and rust susceptibility, root worm resistance, and dropped ears. Inbred 38-11 ears are long, medium thick, and have 16 kernel rows on a red cob with medium length shank. Kernels are small, rectangular crown, yellow wall, slight dent, and show wide channels between kernel rows. Leaves are 86 cm (34 in.) long, 9.5 cm (3.7 in.) wide, medium rigid and medium dark green. Plants are medium tall with high ear height. Tassels have spreading, declined branches with short peduncle. Inbred 38-11 sheds pollen from yellow-green anthers in 78 days. Its yellow-green silks emerge in 81 days.[56]

2. 461

Duddleston's 461 was the pipeline that led to 461-3, B164, and Tr inbreds. It was probably the source of Fe inbred. Troyer Reid variety may have benefited from natural selection in a river-bottom field with subterranean water level that encouraged deeper rooting.[12]

3. A632

Dr. E. H. Rinke, Professor, University of Minnesota, developed A632; it was released in 1963. A632 contributed 15% of total U.S. seed requirements for 1975.[49] Its development began in 1953 at Waseca, MN. when inbred B14 was crossed to Montana inbred Mt42. The single-cross hybrid was selfed during the winter at Homestead, FL. In 1954, 500 plants of the F2 were grown and the 15 earliest silking plants were backcrossed to inbred B14. Final selection was the progeny of the 10 lowest ear-moisture plants. In 1955 the 10 ears were grown ear-to-row. The two earliest plants

in the earliest rows were selfed and also backcrossed to inbred B14 to make the BC2. Only 50 kernels were harvested from the two backcross plants that became inbred A632 because of poor "nick." In 1956 the 1955 procedure was repeated to make the BC3. In 1957 12 ears were grown ear-to-row and the earliest plants in the earliest row were selected.

In 1958 the materials were moved to St. Paul, MN and the BC3S2s were grown in breeding and in observation nurseries and the seven earliest plants in the earliest rows were self-pollinated. Phenotypic selection was for stalk rot and for corn borer resistance. In 1959, 27 ears of BC3S3 were grown ear-to-row in breeding and in observation nurseries. Top crosses were made. Phenotypic selection was based on stalk rot and corn borer resistance. Two ears from one row were saved that eventually became A632. In 1960 the six earliest plants in each row were selfed. Phenotypic selection was for stalk rot and corn borer resistance and for shorter shank length. Five ears were saved from one row and bulked. In 1961 10 plants per row were selfed to make the BC3S5. Fifteen three-way crosses were made. In 1962 10 plants were sibbed and bulked. In 1963, 23 selfed ears were transferred to the Minnesota Crop Improvement Association for release as inbred A632 (see Section II C).

Inbred A632 ears are 14 cm (5.5 in.) long, 3.5 cm (1.4 in.) in diameter, and have 14 kernel rows and medium long (12.2 cm) shanks. Kernels are yellow dent, 9.8 mm long, 7.1 mm wide, 4.9 mm thick with orange side color and yellow cap color. Leaves are intermediate angle, 76 cm (30 in.) long, 7 cm (2.8 in.) wide, and 7 in number above the ear. Plants are 187 cm (74 in.) tall with 68 cm (27 in.) ear height. Tassels are open with 10 semi-lax branches and a short (2 cm) peduncle. A632 sheds pollen from green-yellow anthers in 71.5 days. Tan colored silks emerge in 73.5 days.[57]

4. B164

Mr. Raymond Baker finished B164 from Duddleston 461 in 1930. B164 was a parent of Modern Iodent and Early Iodent inbreds (see Sections II D 2, II D 10, Figure 14.3). B164 contributes high yield, lodging resistance, and smut susceptibility to hybrids. It has a strong two-ear habit. Ears are long, slender, medium sized, covered well, and have red cobs. Kernels are smooth, medium-yellow color, long, irregular shaped with hard starch and deep dent. The plant shows vigorous growth, medium diameter, tall (13 internodes) stalks with long shanks, well-braced strong roots, and medium green, wide, arched leaves. Tassels are large with medium number of long branches. It sheds pollen well. It flowers late for its grain moisture at harvest. B164 sheds pollen from reddish anthers in reddish glumes in 77.5 days. Colorless silks emerge in 80 days.[33,58]

5. B14 and B37

Dr. Sprague developed Iowa inbreds B14 and B37 from Stiff Stalk Synthetic. They were released in 1953 and 1958, respectively. B14 and B37 were 9% and 26% of U.S. seed requirements in 1971. They became the parents of 71 and 27 public inbreds by 1983.[49] They came from the first cycle of recurrent selection of Iowa Stiff Stalk Synthetic (SSS-CO) with double-cross hybrid Iowa 13 as tester. The first test of 169 entries was grown in 1940. Two three-replication locations were planted; yield information was discarded from one location for lack of uniformity. The 10 highest yielding S1 lines, based on test cross, were intermated to continue the selection program.

Inbred B14 evolved from one of the 10 elite S1 lines; it was further tested in top-crosses and in three-way crosses. It contributes vigorous growth, unequaled root and stalk strength, very good yield and fast dry down to hybrids. It is leaf-disease and European corn borer susceptible, but often does well in spite of significant loss of leaf area. B14 ears are medium long, thick, and have 14 or 16 kernel rows, dark-red cobs, and long shanks. Kernels are medium large, and have rectangular crowns, deep-yellow wall color, cup dents, and medium narrow channels. B14 has medium plant and ear height. Leaves are medium rigid, 90 cm (35 in.) long, 10.5 cm (4.1 in.) wide, and deep

green in color. Tassels are spreading, rigid, and have declining branches. B14 sheds pollen from light pink anthers in red glumes in 73 days. Pink silks emerge in 78 days.

Inbred B37 was *not* derived from one of the 10 intermated S1 elite lines. B37's progenitor was the second highest yielding of 169 entries, was in the best 5% for lodging, had no dropped ears, and was near average for ear height, grain moisture, and percent stand in the 1940 test-cross tests.[59] However, concern existed about meager pollen shed and about silk delay. It was difficult to increase. Consequently, it advanced at a slower pace. Its high yield and good kernel quality became more apparent in single-cross hybrids, particularly at higher fertility and at higher plant densities. Successful foundation seed increases usually had two dates of planting and harvested only the first planting date. It made excellent hybrids with OH43 and C103 derivatives.

Inbred B37 ears are 12 cm (4.8 in.) long, 3.3 cm (1.3 in.) in diameter, and have 10 kernel rows, red cob, and medium long (8 cm) shanks. Kernels are 7.7 mm long, 7.7 mm wide, and 7.0 mm thick with orange side and cap color. Leaves are 20° angle, 64 cm (25 in.) long, 10 cm (4 in.) wide, and six in number above the ear. Plants are 196 cm (77 in.) tall with 78 cm (31 in.) ear height. Tassels are semi-compact with seven branches and medium (5 cm) peduncles. B37 sheds pollen from tan-red anthers in 77 days. Green-yellow silks emerge a week or more later.[60]

6. B64 and B68

Drs. L. H. Penny and F. F. Dicke developed B64 and B68 at Iowa State University.[61] Both inbreds were released in 1968. They selected B64 and B68 from F2 and backcross populations of 41.250B × B14 under heavy European corn borer infestations. B68 is a vigorous inbred, similar to B14 in plant and ear type, but flowers 4 to 5 days later. It has considerably more tolerance to leaf blights than B14. In hybrids, B68 is at least equal to B14 for yield and for root and stalk strength, but has later maturity.[61] B64 is stress tolerant. B68 goes barren under stress.

Inbred B64 ears are 15 cm (6 in.) long, 3.3 cm (1.3 in.) in diameter, and have 12 or 14 kernel rows and white cobs with medium long (8 cm) shanks. Kernels are yellow dent, 8.3 mm long, 7.8 mm wide, and 5.4 mm thick with orange side and cap color. Leaves are 37° angle, 86 cm (34 in.) long, 8 cm (3.2 in.) wide, and number five above the ear. Plants are 214 cm (84 in.) tall with 89 cm (35 in.) ear height. Tassels are open with eight, rigid branches and a short (4 cm) peduncle. B64 sheds pollen from tan anthers in 82.5 days. Purple silks emerge in 87.5 days.[57]

Inbred B68 ears are 16 cm (6.4 in.) long, 3.7cm (1.5 in.) in diameter, and have 14 or 16 kernel rows and red cobs with medium long (11 cm) shanks. Kernels are yellow dent, 9.3 mm long, 7.7 mm wide, and 4.4 mm thick with brown side color and orange cap color. Leaves are 49° angle, 88 cm (35 in.) long, 7 cm (2.8 in.) wide, and number six above the ear. Plants are 209 cm (82 in.) tall with 83 cm (33 in.) ear height. Tassels are open with nine rigid branches and a short (4 cm) peduncle. B68 sheds pollen from green-yellow anthers in 82.5 days. Red silks emerge in 87.5 days.[57]

7. B73

Dr. Wilbert A. Russell, Professor, Iowa State University developed B73 from Stiff Stalk Synthetic. It was released in 1972. B73 was 16% of total U.S. seed requirements in 1980. B73 came from cycle 5 of GCA recurrent selection of Stiff Stalk Synthetic (BSSS-C5), using double-cross hybrid Iowa 13 as a tester. Cycle 5 test crosses were tested at four locations in 1962. Ten S1 lines were selected by Dr. Lowell H. Penny in Cycle 5 to make Cycle 6. He gave Dr. Russell S2 seed of the selected lines for further inbreeding and selection in 1964. Inbred B73 was developed from one of these 10 S2 lines. Typically, two ear rows were grown from each selected previous ear row each year in the breeding nursery. Phenotypic selection for synchronous flowering was practiced each generation. Usually 8 to 10 plants were self-pollinated, and seed was saved from two plants in one selected ear row. Dark green color, upright leaves, and large ear size made B73 unique in the nursery. At the same time, top cross evaluation of the S2s and S3s were on a double-double (8 inbreds) hybrid tester, then single cross evaluation of the S4s and S5s with

four inbred-line testers (including inbred Mo17) followed. Top cross evaluations were usually based on two or three locations, and single cross tests at three to five locations. The inbred showed outstanding hybrid performance that kept it alive in spite of below average European corn borer tolerance. It was marked for discard one year, but fortunately the seed remained after hybrid yield test results were calculated.[60] Hybrid B73 × Mo17, first grown commercially in 1973, was particularly outstanding; it soon became a very popular hybrid. B73 also made excellent hybrids with OH43 derivatives.

Inbred B73 ears are 14 cm (5.5 in.) long, 4.3 cm (1.7 in.) in diameter, and have 16 or 18 kernel rows, red cobs, with medium-long (7.6 cm) shanks. Kernels are yellow dent, 11.3 mm long, 7.2 mm wide, 4.1 mm thick with orange side color and yellow-orange cap color. Leaves are 30° angle, 74 cm (29 in.) long, 9 cm (3.5 in.) wide, and number six above the ear. Plants are 212 cm (83 in.) tall with 92 cm (36 in.) ear height. Tassels are compact with six to eight upright branches and medium (7 cm) peduncle length. B73 sheds pollen from tan-pink anthers in 75 days. Green-yellow silks emerge in 78 days.[57]

8. C103

Dr. Donald F. Jones developed inbred C103 from Lancaster Sure Crop at the Connecticut Experiment Station. It was released in 1949. Ears were collected at the Noah L. Hershey farm in Chester County near Parkesburg, PA in 1938. Jones did ten generations of individual plant and progeny selection. He states C103 stands erect until the end of the season, free from stalk breakage and premature death. Its stalk has a large number of vascular bundles, heavy walled fiber cells, and solid pith cells with high sugar content. He describes C103 in hybrids as not only high yielding but also low in number of root lodged and stalk broken plants that are relatively free from stalk and ear diseases. C103 also contributes clean leaves, long husks, and poor tolerance to drought.[62] Inbred C103 was a parent of DEKALB 805 the first popular, widely grown, single-cross hybrid.

Inbred C103's ears are long, medium thick, and have 12 kernel rows, dark red cobs, long tight husks, and medium long shanks. Kernels are large, rectangular shaped crown, rust colored wall, light dent, and show medium channels between rows. Leaves are 83 cm (33 in.) long, 10.5 cm (4.1 in.) wide with medium rigidity and medium greenness. Plants are medium tall with slender stalks, long internodes, and medium ear height. Tassels are spreading with rigid branches and medium long peduncle. C103 sheds pollen from pale-green anthers in 74 days, Pink silks emerge in 78 days.[57]

9. F2 AND F7 (FRANCE)

Before World War II, corn for grain was grown only in a few regions of France and totaled 4000 to 4500 hectares. In southeastern France, about midway between Toulouse and Montpelier, lies the town of Angles and near it the La Capte farm located in a remote, hilly area where the climate allows only marginal farming. The location is 43.5° N lat at 950 m (3116 ft) elevation. After decades of abandonment, the Henri Primo family occupied the La Capte farm. In later years, Henri lived there alone, almost a hermit. Each year he grew a small patch of forage corn for his cows. He obtained seed (variety not stated) from the grocery store in Angles. Because of the cool, short season the corn was not expected to mature, but one year one plant produced mature seeds. Henri harvested the seeds and planted them the following year and repeated the process for the following several years. He developed a variety that was very early and cold tolerant.

After the Liberation, the Marshall Plan to help rebuild Europe brought recent hybrid corn technology to France. The INRA (National Institute for Agronomic Research) Plant Breeding Station at Versailles developed the objective of introducing corn to all regions of France. Mr. Andre Cauderon directed the project, and Mr. Roger de Larambergue was a member of the corn team that began collecting corn populations. In 1946 during a vacation in Angles, Larambergue

saw some vigorous corn growing in his father's vegetable garden. Henri Primo had provided the seed. Larambergue named the variety Lacaune (a larger, better known, nearby town) and took a packet of seed to Versailles where Cauderon planted it in 1947. The first generation from Lacaune variety provided only ten desirable, self-pollinated plants, but the following generations provided larger harvests.[63]

Cauderon finished inbreds F2 and F7 in 1955. They were first used in INRA200 (WH.WJ × F7.F2), which was launched in 1957. Inbreds F2 and F7 have enjoyed great success — particularly as partners of American inbreds. Additional popular hybrids include INRA258, INRA260, Limagrain LG11, Cargill Primeur 170, Pioneer DEA, DEKALB DK250, and others. Inbreds F2 and F7 were popular so long that France changed its patent law in the late 1960s to collect royalties for additional years. Today, more than 3 million hectares of corn are grown annually in France (see Table 14.3).

Inbred F2 ears are 12 cm (4.7 in.) long, 3.3 cm (1.3 in.) in diameter, and have 12 kernel rows, white cobs, and short (6.5 cm) shanks. Kernels are yellow flint, 7.1 mm long, 7.2 mm wide, and 5.7 mm thick with deep-yellow side color and orange cap color. Leaves are upright, 65 cm (26 in.) long, 7 cm (2.8 in.) wide, and four in number above the ear. Plants are 150 cm (59 in.) tall with 54 cm (21 in.) ear height. Tassels are compact with eight upright branches and a short (4 cm) peduncle. F2 sheds pollen from tan anthers in 66.5 days. Red silks emerge in 68 days.[57]

Inbred F7 ears are 13 cm (5.1 in.) long, 3 cm (1.2 in.) in diameter, and have 12 kernel rows, white cobs, and medium long (8 cm) shanks. Kernels are yellow flint, 8.3 mm long, 7.5 mm wide, and 4.9 mm thick with orange side and cap color. Leaves are 40° angle, 70 cm (28 in.) long, 7 cm (2.8 in.) wide, and number four above the ear. Plants are 129 cm (51 in.) tall with 48 cm (19 in.) ear height. Tassels are compact with two or three branches and a very short (1 cm) peduncle. F7 sheds pollen from tan-yellow anthers in 62 days. Green-yellow silks emerge in 64.5 days.[57]

10. I205

Dr. Merle Jenkins developed I205 from Iodent Reid. It was released in 1937. It transmits high yields, erect plants, good ear retention, and tolerance to smut, northern leaf blight, European corn borer, and chinch bugs to hybrids. Its ears are susceptible to molds, and being thick may dry slowly. It is preferably used as a male parent. One of its first good hybrids was with inbred L289.

Inbred I205 ears are medium thick, medium long, and have 16 kernel rows, dark red cobs, and short shanks. Kernels are medium large, rectangular crown, deep yellow wall color, deep dent, and show narrow channels between kernel rows. Leaves are 81 cm (32 in.) long, 9.5 cm (3.7 in.) wide, medium rigid, and medium green color. Plants are medium short with medium high ear height. Tassel is diffuse with drooping branches. I205 sheds pollen from pink anthers in glumes with a trace of red in 72 days. Yellow-green silks emerge in 73 days.[56]

11. MO17

Drs. Marcus S. Zuber and Clarence O. Grogan developed MO17 at the University of Missouri. It was released in 1964. It resulted from Grogan's tester-choice thesis study. The parental population of interest was 44 selected S1 plants from hybrid C103 × 187–2. The selection objectives were higher yield from longer ears from both parents, more leaf-blight and stalk-rot resistance from inbred C103, and better roots, better seed quality, and faster drying from inbred 187–2. The three testers for these 44 S1s were hybrids U.S. 13, WF9 × 38-11, and L317 × Hy. Segregate 889–7 had the highest yield, the lowest stalk breakage, and average ear height. Progeny of 889–7 were grown ear-to-row and phenotypically selected. Five inbreds were test crossed to testers that included Iowa inbreds B14 and B37 and evaluated by the University of Missouri and by Missouri Farmers Association Seeds. Line 4524 became inbred MO17.[64] It has resistance to leaf diseases, smut, and

common rust. It has poor cold germination test and poor shelf life. It flowers early for its harvest kernel moisture. Its first popular hybrid was with Nebraska inbred N28 in 1968 followed by a very popular hybrid with Iowa inbred B73 in 1973. Its most popular short-season hybrid was with Minnesota inbred A634 first sold in 1973.

Inbred MO17 ears are 18.3 cm (7.2 in.) long, 3.5 cm (1.4 in.) in diameter, and have 10 kernel rows, red cobs, and long 12.2 cm (4.8 in.) shanks. They have high shelling percentage and good test weight. Kernels are yellow dent, 10.3 mm long, 8.5 mm wide, 4.7 mm thick with yellow side color, and orange cap color. Leaves are 36° angle, 67 cm (26.4 in.) long, 9 cm (3.5 in.) wide, medium green color, and number five above the ear. Plants are 208 cm (82 in.) tall and ear height is 84 cm (33 in.). Tassels are compact with rigid branches few (6) in number and a long (9 cm) peduncle. It has a long silk channel. MO17 sheds pollen from green-yellow anthers in 76 days. Pink-tan silks emerge in 82 days.[57]

12. OH43

Mr. Glenn H. Stringfield developed inbred OH43 from the hybrid OH40B × W8 at the Ohio Experiment Station. OH40B was developed from a Richey Lancaster Synthetic. W8 is 50% Minnesota 13 variety and 50% Northwestern Dent variety. OH43 was released in 1949. He describes it as contributing resistance to destructive wind, drought, insects, and diseases, and that OH43 is usually among the best for production of high yields of sound corn on standing stalks. He recommends it as a seed parent. But, it may leaf out underground causing poor stands. It flowers very early relative to its kernel moisture at harvest. Ear dry down in the field is slow.

Inbred OH43 ears are medium long, medium thick, and have 14 kernel rows, white cobs, tight husks, and medium short shanks. Kernels are yellow dent, medium size, rectangular-shaped crown, slight dent, yellow wall color, and show narrow channels between kernel rows. Leaves are medium rigid, 76 cm (30 in.) long, 10.5 cm (4.1 in.) wide, and medium green in color. Plants are short with low ear height. Tassels are spreading with rigid branches and medium peduncle length. OH43 sheds pollen from yellow-green anthers in 68 days. Yellow-green silks emerge in 73 days.[56]

13. OH07

Mr. Glenn H. Stringfield developed inbred OH07 from hybrid CI540 × L at the Ohio Experiment Station. CI540 is from Illinois Two Ear and Illinois L is from Leaming. OH07 was released in 1943. It combines well with B14 and B73 derivatives. He describes it as having a strong two-ear habit, excellent stalk quality, and drought tolerance; thus, its hybrids have a high tolerance to high plant density. OH07 contributes resistance to European corn borer, virus, and smut. It has good ear dry down qualities. It is relatively weak rooted, susceptible to leaf blights, and grows slowly in the seedling stage. It flowers late relative to its kernel moisture at harvest. HY2 × OH07 was one of the first hybrids to show genetic tolerance to higher plant densities.

Inbred OH07 ears are medium long, slender, somewhat flattened, and have 14 to 16 kernel rows, red cobs, and medium long shanks. Kernels are medium size, rectangular crown, cup dent, deep-yellow wall color, and show narrow channels between kernel rows. Leaves are upright, medium rigid, 78 cm (31 in.) long, 9 cm (3.5 in.) wide, and dark green in color. Plants are tall and have medium ear height placement. Tassels are spreading and have rigid branches with medium long peduncle. OH07 sheds pollen from yellow-green anthers in 78 days. Pink silks emerge in 81 days.[56]

14. OS420

Dr. Merle T. Jenkins developed OS420 from Osterland Reid at Iowa State College. It was released in 1937. It became a parent of popular double-cross hybrid Iowa 939. It contributes good root

anchorage, poor stalk quality, and some tolerance to insects and diseases. Thick hybrid ears may dry slowly.

Inbred OS420 ears are long, medium thick, cylindrical, and have 14 kernel rows, light-red cobs, and medium length shanks. Kernels are large, round crown, cup dent, light-red walls, and show medium channels between kernel rows. Leaves are medium rigid, 86 cm (34 in.) long, 9.5 cm (3.7 in.) wide, and medium green in color. Plants are medium tall with medium ear height. Tassels are diffuse, have rigid branches and medium length peduncle. OS420 sheds pollen from light pink anthers with a trace of red in the glumes in 70 days. Yellow-green silks emerge in 73 days.[56]

15. W64A

Dr. Norman P. Neal developed W64A from hybrid WF9 × 187–2 at the University of Wisconsin. It was released in 1954. It was a parent of DEKALB XL45 and Michigan 300 (OH43 × W64A) a very popular 110RM hybrid. It was second only to B37 in the 1970 ASTA most widely used inbreds survey. It contributes early flowering, average plant and ear height for its maturity, very strong roots, and good stalks. Inbred W64A ears are 13 cm (4.5 in.) long, 4.2 cm (1.7 in.) thick and have 16 kernel rows, white cobs, and medium long (8 cm) shanks. Kernels are 9.3 mm long, 6.6 mm wide, and 4.6 mm thick with orange side and yellow cap color. Leaves are 40° angle, 64 cm (25 in) long, 9 cm (3.5 in.) wide, and 6 in number above the ear. Plants are 167 cm (66 in.) tall with 54 cm (21 in.) ear height. Tassels are compact with 6 branches and medium (7 cm) peduncles. W64A sheds pollen from green-yellow anthers in 69 days. Green-yellow silks emerge in 72 days.[57]

16. WF9

Mr. Benjamin H. Duddleston developed WF9 (Wilson Farm row 9) from Reid Yellow Dent at Purdue University. It was released in 1936. In 1935 it became one parent of hybrid U.S. 13, the first nationally popular double-cross hybrid. In 1942 WF9 was grown on 93% of 7000 ha (17,500 acres) of seed corn production of public hybrids (24 of 27) for the five central U.S. Corn Belt states.[53] In the early 1960s, it was a parent of a popular, single-cross hybrid commercially produced by many smaller companies for more than a decade. WF9 was probably grown in hybrids on 30% or more of U.S. corn acreage for 30 years; making it the all time, most popular inbred. Wilson Soils and Crops Research Farm (Wilson Farm) was the Purdue Agronomy Farm from 1913 to 1950, located at the junction (southeast quadrant) of highways IN26 and U.S.52 just east of Lafayette, IN (NE 1/4 of Section 27 in Fairfield Township, Tippecanoe County, IN).[12] WF9 contributes good root anchorage, medium good stalk quality, lush vegetation, and excellent seed size and shape for mechanical planters to its hybrids. It is susceptible to leaf blights, corn borers, aphids, and ear mold. It is a poor pollen shedder.

Inbred WF9 ears are medium long, medium thick, and have 16 kernel rows, dark red cobs with medium long shanks. Kernels are medium large, rectangular crown, deep-yellow wall color, deep dent, and show narrow channels between rows. Tassels are diffuse, have drooping branches, and medium length peduncle. Plants are medium height with medium low ear height. WF9 sheds pollen from yellow-green anthers with a trace of red on the glumes in 72 days. Yellow-green silks emerge in 73 days.[56]

F. Useful Synthetics

1. Iowa Stiff Stalk Synthetic (BSSS)

Dr. George F. Sprague intermated 16 inbreds (two were second cycle; therefore, 18 total) of above average stalk quality in the early 1930s. Ten of the 16 inbreds had Reid Yellow Dent background, and some of the remaining six have unknown or partially unknown backgrounds that may contribute

more Reid Yellow Dent. Hence, Iowa Stiff Stalk Synthetic belongs to the Reid Yellow Dent heterotic group.[49,65] Iowa Stiff Stalk Synthetic (SSS) has been a consistent contributor of Reid Yellow Dent germplasm to U.S. Corn Belt hybrids for the past four decades.

Iowa Stiff Stalk Synthetic underwent selection experiments for population improvement by the corn-breeding project at Iowa State. The project developed several useful inbreds including: B10, B14, B37, B73, and B84. The more popular of these inbreds were B37 (26% of total U.S. seed requirements for 1971), B73 (16% of total U.S. seed requirements for 1980), and B14 (9% of total U.S. seed requirements for 1971). Inbreds B37, B73, and B14 were the parents of 27, 14, and 71 second-cycle, public, inbreds, respectively, by 1983.[49] Inbred B14 is the parent of early inbreds for the northern U.S. Corn Belt, for Canada, and for Europe. They include A632, A634, A635, A640, and A641 developed at the University of Minnesota by the Rinke method, and CM105 developed by Dr. Johan (John) Giesbrecht at Morden, Manitoba.[32] Inbred A632 contributed 15% of total U.S. seed requirements for 1975.[49] Iowa Stiff Stalk Synthetic is about 8% of U.S. hybrid corn background as derivatives of inbreds B14, B14A, B37, B64, B68, and B73 (Figure 14.2).[12]

Iowa Stiff Stalk Synthetic and early generation inbreds from it were developed and test crossed at Ames, Iowa. Test crosses were grown at a total of 19 locations from 1940 (C0) through 1965 (C6), including six at Ames and four at Ankeny in central Iowa, three in east central, three in west central, two in southeast, and one in southwest Iowa.[66]

2. Pioneer Prolific Composite (PRC) a.k.a. Iowa BS11

Dr. W.L. Brown and Mr. Karl H. Jarvis of Pioneer Hi-Bred Int'l. developed PRC. It is a prolific composite made up of nine open pollinated varieties and two semi-prolific, experimental, U.S. Corn Belt hybrids. The varieties were Argentine Pop, Caraway Pop, Clark Yellow Dent, Golden Prolific, Jarvis, Mexican June, Mosby Prolific, Neal Paymaster, Turkish Prolific Pop, and Whatley Prolific. The earliest inbred in the experimental hybrids was a B164 derivative.[4] Because of the southern origin of much of the background, they enforced strong selection for lower plant and ear height (adaptedness) and for yellow endosperm. They selected for multiple ears on the main stem and against tillering of plants grown in isolated plots. They removed or detasseled undesirable plants before flowering. Selection continued near Johnston, IA for about 20 years. Pioneer Hi-Bred International gave PRC to Iowa State University, who designated it Iowa BS11. SMPR in Figure 14.2 is PRC Cycle 5 that was selected five generations for early flowering (55 of 1100) among plants grown at high plant density at Mankato, MN.[4,67]

Weyhrich et al.[68] used Iowa BS11 as source material for comparing four or more cycles of seven different (six intra- and one inter-population) recurrent selection methods. For each method, they determined the relative effectiveness and relative cost for improving the genetic potential of this germplasm.

G. Summary and Conclusions

Adaptation is the sole driving force of evolution. Better adaptation to the environment means higher yield. Knowledge of previous geography and climate where a corn genotype was adapted, and how it was selected, helps the breeder select useful germplasm for breeding.

Reid Yellow Dent benefited from the mixing of early and late U.S. Corn Belt adaptedness genes. Iodent variety was Reid Yellow Dent selected for earlier flowering that provided better adaptedness to Iowa. Iowa and Minnesota rarely obtain enough rainfall in July and August; stored subsoil moisture saves the corn crop. Earlier flowering helped avoid drought. Cumulative selection (Figure 14.3) not only advanced the evolution of corn but also provided commercially useful inbreds at each step of the process. Lancaster Sure Crop is a flint that was repeatedly crossed to dents to increase its yield, and then selected for smooth, flinty ears. The flint kernels would be poor for feeding, but it may have been popular for milling (larger grits). Leaming Corn was selected for

early ripening, but was forced to yield well in a long-season area. It was selected in the northern part of the first, southern U.S. Corn Belt as corn growing was moving north and west. Minnesota 13 was selected for a greater number of mature dent ears in a short-season area. Northwestern Dent was selected in a very severe, short season, droughty, long day, temperate environment. It became very stress tolerant. Again, early and late U.S. Corn Belt adaptedness genes were mixed, and dent corn germplasm was made earlier.

Corn breeding progress (increased yield) in the U.S. Corn Belt and in Europe over the past 100 years has come largely from replacing flint with dent (Reid Yellow Dent) germplasm. Reid Yellow Dent has been the primary dent germplasm used to introgress and to replace flint corn. Nevertheless, Northern flints have remained an important source of seedling vigor and of cold tolerance for the cool springs. They are also a source of hard kernels for combining high moisture corn in western and northern Europe and of earlier flowering for the short season in Canada, northern Europe, and northern Japan. South American flint types in Argentina and in Brazil are presently being introgressed by dent germplasm.

III. BEHAVIOR OF CORN

A. Cross-Pollinated, Monoecious Species with Imperfect Flower

Cross-pollinated species carry a genetic load of deleterious recessive genes that are routinely purged by natural selection from homozygous self-pollinated species. Cross-pollinated species usually show more heterosis and more variation than self-pollinated species. For these reasons, breeding methods differ between self- and cross-pollinated crops. Breeders trained for self-pollinated crops may be more adept at developing inbreds, but may benefit from training in quantitative inheritance and in combining ability. Corn inbreds are unnatural entities. Test crosses are necessary to evaluate lines because of imperfect inbred-hybrid correlation.[69]

Separated male and female flowers on the same plant make hand pollination easy with hundreds of kernels expected per attempt (Table 14.5). These procedures have been taught to tens of thousands of pollinators of high school age. Separated male and female flowers make emasculation (detasseling) for seed production relatively easy by mowing, wheel pulling, or hand pulling, and both cytoplasmic and genetic male sterility are available. The many-seeded ear is convenient for harvesting and for drying seed corn. The number of seeds harvested per seed planted, in the hundreds for seedsmen and often exceeding a thousand for farmers, coupled with relatively low planting rates, provide the favorable business economics for the seed corn industry. The relatively large seed size aids accurate planting and also aids emergence in variable soil moisture conditions.[69]

Corn is an efficient plant. It is a warm season crop with a high net assimilation rate due to relatively low photorespiration that is associated with C-4 photosynthesis (photosynthesis first forms a four carbon atom chain). Most other crops, except for sorghum, have C-3 photosynthesis with higher photorespiration, thus lower net assimilation rates. Size of plant, plant morphology, and length of stay-green limit photosynthetic capacity.[69]

B. Planting Corn

The key to planting straight rows of corn is accurate turning radius at the ends of the field. If the rows are bowing in at the ends, the turning radius is too short. If the rows are bowing out, the turning radius is too long. Practice in a parking lot. The center of the planter must move exactly the number of planter rows times the distance between rows.

It helps to have a sighting rod on the front and center of the tractor; it should extend above the operator's line of sight. A tape down the top center of the tractor will help the operator sight from the center of the tractor. The corn planter must be centered on the tractor.

TABLE 14.5
Instructions for Pollinating Corn

Shoot bagging

Look for plants where tassel is showing from whorl — ear shoots will start to emerge in a short time. When ear shoot is 1 to 3 cm long and before silks have emerged, slide glassine bag over shoot, seam side (long flaps) toward stalk. Do not remove ear leaf. Pull bag down and around stalk so flap is pinched between shoot and stalk. Staple lower corners around stalk if large shoot bags permit. As plants and shoots grow, they will tend to push shoot bag off, so be sure to snug shoot bag down over shoot. Pull down shoot bags that have worked loose on plants previously bagged. Shoot bag topmost shoot unless instructed otherwise. Be sure top shoot is bagged from previous days and move shoot bag up to top shoot if necessary.

Putting up bags

Must have pollen — live tassel with extended plump anthers; must have silks — do not expose or touch silks. Slide hand over tassel to remove old anthers. Hold tassel in left hand, slide bag (with seam away) over tassel with right hand; one or two leaves may be included if short tassel. Fold up corners, then fold over and secure bag with paper clip. Bottom of bag should fit tightly around peduncle. Bags should be up overnight before pollination.

Taking bags down

Must have good pollen — good pollen is bright yellow and falls freely from anthers on the tassel. With average temperatures, pollinations should be made between 9:30 a.m. and 3:30 p.m. CDST. Bend plant over until bag is beyond horizontal. Rap bag once or twice sharply. Remove paper clip and shake bag while sliding it off tassel. Allow pollen to slide to bottom of bag. Fold bag in half and make funnel of open end (seam outside of fold). Do not place hand inside tassel bag. Do not remove ear leaf. Slide of bag — under cover of funnel of tassel bag. If silks are very long they may be shortened by grasping shoot bag tightly and twisting as you slide off shoot bag. Do not allow silks to be exposed. Unfold tassel bag and shake bag to cover silks with pollen. Seam should be toward plant. Wrap inner corners of tassel bag around stalk and secure with one staple 10 or 12 cm up from bottom of bag.

Experienced farmers and corn breeders plant corn deeper than do their novice peers. Nonirrigated fields planted too shallow will have poor stands in dry springs. My minimum depth of planting is 5 cm (2 in.), then follow the moisture down. It is imperative to plant in moist soil. I have planted several nurseries at depths of 10 cm (4 in.) or deeper and attained satisfactory stands. The permanent root system develops from the lower three or four nodes of the stem and is independent of planting depth. The length of the coleoptile determines the depth of the growing point. The growing point remains underground until the corn is about 30 cm (12 in.) tall.[29]

I am a strong advocate of wire-cone planters. They allow easy choice of plant density and good distribution. A constant speed is necessary (exactly 5, 6, 7, or 8 seconds between buttons). Slower speeds cause more mistakes (double planting). A tighter wire makes straighter alleys. Adjusting the fork mechanism to center the button in the alley is an exacting process. Tie up the press wheel next to the wire and plant on top of the ground. Use full packets of dead seed (microwave) to ensure a kernel in each cell of the cone. Several trial runs of 10 to 12 buttons at the correct speed are necessary. Measure carefully. Walk beside the unit planting and set a pot label where the first and the last kernel hit the ground. Discard outliers, then calculate means fore and aft of the button to corn. Adjust the fork. Set the other fork by measuring from the tool bar. It is necessary to check the fork setting when changing fields or as the soil changes moisture content. The most common and most serious planting mistake is starting on the wrong button. Mark the first button and the button *following* the last range with bright tape.

I plant corn nurseries as soon as, or before, the first farmers plant corn in the area — certainly among the first 10%. I encourage you to plant your nursery *before* planting yield trials. In Minnesota it often required 3 weeks or more for my nurseries to emerge. In northern Illinois they usually

emerge in 14 to 17 days. Early stand count percentage (emerged/planted) in nurseries and in yield test fields is an important selection trait to help provide better stands for the farmer. Earlier planting increases yield, decreases plant height, and decreases lodging in the farmers' fields. The earlier of two plantings may decrease plant height and stalk breakage even when both plantings emerge at the same date. Things are going on underground. Cooler temperatures during early development shorten internodes.

Seed coat integrity (to keep fungi out) is important to cold test germination. Damage to the seed coat during harvesting and conditioning reduces cold test germination. Seed from higher yielding (larger kernels) fields may be more susceptible to damage because their seed coats (female tissue) have been stretched thinner. Flat kernels protect the germ face (embryo) more than round-shaped kernels. A warm test predicts germination in warm springs; a cold test predicts germination in cold springs. Neither predicts the other.[69]

Angle of secondary root growth is a heritable trait, but corn roots grow only where environmental conditions permit. Seedbed temperatures warm from the surface down. Cooler soil temperatures promote more horizontal secondary root growth, thus reducing early root lodging. Cooler spring soil temperatures in Europe cause vertical-rooted inbreds to develop more horizontal secondary roots. French inbreds F2 and F7 root lodge much more in the U.S. than in France for this reason. Late planting (warmer soil) of an alternate nursery can be useful for increasing the incidence of root lodging for phenotypic selection because more vertical secondary root growth occurs.

Crusting reduces stands and increases rainfall runoff. Crust formation follows breakdown of soil aggregates by excessive tillage or by raindrop impact. Clay particles fill the smaller voids in silt and sand causing a closer packing (increased bulk density) similar to paste filling smaller voids in sand and gravel to form concrete. Alluvial soils more often have a range of particle size conducive to crusting. Less organic matter also increases crusting. Drying of the wet, homogenized soil causes shrinkage and crusting. Crusts may persist with rainfall if cool temperatures slow plant growth. Avoid overworking the seedbed. If crusting occurs, rotary hoe as soon as possible. Plastic stakes (large pot labels) withstand rotary hoeing and can be reused several years.[70]

Shrinking of the clay fraction as the soil dries causes corn planter furrow opening. Do *not* plant in a wet seedbed. Use the mud ball test. If the ball crumbles, the soil is dry enough to work and to plant. Deeper planting will lessen the problem. More down pressure on the press wheels may help. The problem may be soil type specific.[70] Talk to your neighboring farmers about planting corn; seek their advice. You will probably learn something useful.

C. Plant Density Response

Corn plant densities have been increasing as long as records have been kept. Generally, they have increased along with fertilizer rates. But for the last two decades in the U.S., nitrogen rates have leveled off at 130 kg/ha (130 lb/acre) while plant densities have continued to increase.[71]

Typically, smaller plants withstand crowding better than larger plants. Earlier, smaller hybrids make their maximum yields at higher plant densities than later, larger hybrids. Leaf area index is more important than plants per unit area for total water requirement. Earlier hybrids have less stalk breakage, root lodging, ear droppage, and silk delay and have fewer barren plants than later hybrids at a given density because they incur less stress.[10] However, inherent responses to plant density, independent of plant size, exist. Corn differs in response to higher plant density because of tolerance to shortages of sunlight, moisture, and fertility. Cultural practices, such as increased plant density and narrower rows, can help short hybrids intercept more light in total and more light per plant to compensate for their smaller plant size.[9,19]

Higher plant densities for corn performance testing increase the range of performance making selection easier for yield, broken stalks, and dropped ears in small plot 1250 per hectare (500 per

acre) testing. Higher plant densities reduce the number of strip test plots 8 per hectare (3 per acre) by more than half to differentiate two hybrids of interest for yield, broken stalks, and root lodging.[19]

How high is high? I use twice the plant density of local, progressive farmers for nursery selection. This isn't as high as it sounds because inbreeding reduces plant size. For hybrids, I recommend 20% more than state average for normal and at least 30% more plants for highest plant density in yield testing. It takes several years to develop a hybrid; meanwhile, plant densities continue to increase.[71–73]

D. Seedling and Juvenile Plant Growth

Seedling vigor is important. During the first 4 to 6 weeks of growing season, seedling vigor is the farmer's basis for hybrid comparison. It affects sales. Also, fast early growth increases leaf canopy for photosynthesis during longer days. The greatest quantity and best quality of sunlight is on June 21 (summer solstice in the Northern Hemisphere). Early Northern flint varieties have the fastest seedling and juvenile plant growth because of their longer period of adaptation to cooler, higher latitudes. They have migrated a greater distance from their tropical origin, thus better adapting to longer day length, to cooler minimum temperatures, and to wider temperature fluctuations. Seedling vigor scores are an estimate of green mass; they are taken 6 to 9 m (20 to 30 ft) away from, and in line with, the row scored to obtain a better profile. The scores should be taken before the plants are 25 cm (10 in.) tall when they start to predict final plant height. Robust seedlings are advantageous over thin, grassy seedlings because they have more leaf area for photosynthesis.[10,74]

Tillers are lateral branches formed at the base of the plant. Tiller frequency in corn correlates negatively with plant density and positively with favorable growing conditions like other grasses. But, too much time passes between tiller initiation and flowering (when stress most likely occurs) for good self-adjustment to growing conditions. Corn breeders select against tillering to better allow choice of plant (stem) density by the farmer. In spite of long-time selection against tillering, tillers may occur during very favorable growing conditions. Farmer complaints about excessive tillering in the spring seldom result in complaints about deficient yield in the fall.

Herbicides can hurt corn. Inbreds and hybrids that are susceptible to either a normal rate of a popular corn herbicide or to carry-over of a popular soybean herbicide should be discarded. There is danger we may run out of corn before they run out of chemicals. Farmers will change herbicides for a good hybrid in the U.S. Corn Belt; but they are less likely to change herbicides in the southern or in the far western U.S., where corn is the second to fourth ranking crop.

E. Leaf Angle Modification

Corn plant architecture is a Christmas-tree shape that is ideal for light interception. Leaf area index can be easily modified either by flowering time (maturity) of the corn or by the number of plants per unit surface area. Leaf angle can be modified either by simply inherited liguleless genes or by quantitatively inherited factors.[10]

Upright leaves became fashionable for corn along with narrower rows, higher plant densities, better herbicides, and more nitrogen use. Upright leaves cause less self-shading. When sunlight is limiting yield in cool, moist, higher-yielding years, upright leaves may increase yield (more photosynthesis). When water is limiting yield in hot, dry, lower yielding years, upright leaves may decrease yield (more heat and drought stress). However, some upright leaves (strong midrib) are more likely to leaf roll (self-shade) during drought and reduce transpiration.[74] Restrictions on herbicides for environmental protection may bring back lax leaves to better shade the ground, thus reducing weed growth.

Upright leaves are strongly associated with downright roots in inbreds B73, C103, and OH07 and many of their derivatives; they have upright leaves and often root lodge (weak, vertical roots). Luther Burbank states, "the root system, if it could be entirely exhumed, would be very like the

superstructure of the tree."[75] It also works for corn. Specifically, leaf angle to the stem and secondary root angle to the stem are similar. In the central and western U.S. Corn Belt, a negative correlation between root lodging and yield often exists because of the importance of subsoil moisture to yield. Root lodging resistance results from wider, horizontal roots; yield from subsoil moisture results from narrower, deeper, more vertical roots. Early root lodging is most likely to occur during wind and rainstorms just before flowering when corn is in an awkward (top-heavy) stage. Late root lodging occurs near harvest time due to root rot.

Only young root hairs are functional at absorbing water. If heavy, clay soil and subsoil are dry at flowering, rains late in the flowering period may not penetrate the soil level to where young roots are functional in time to reduce moisture stress.

F. Leaf Diseases

My experience with leaf diseases involves both separate disease nurseries and also artificial inoculation in our main nursery. Either will work. The ideal situation is a river-valley-bottom or former lake-bottom field surrounded by hills and trees to reduce winds and air drainage. I believe in early and frequent inoculation. We typically inoculate after each rain, with the first inoculation at or even before seedling emergence. We inoculate until lesions form or we run out of inoculum. Our leaf diseases often remove 30% of leaf area before flowering — this prevents escapes. Moderate leaf disease tolerance is sufficient.

We inoculate with all indigenous leaf diseases. Leaf refuse has the advantage of containing additional, newer disease organisms. The leaves should be allowed to dry in large mesh bags before grinding. We use a hammer mill with an oat screen to grind the leaves. Most livestock growing areas have portable feed grinders on trucks that will come to the field.

Mr. Francis Garing, senior station manager, modified an Ezee-Flow fertilizer applicator with caps and funnels to conserve refuse by dropping ground leaf refuse directly over each row. He mounted the spreader (wheels removed) on a three-point hitch with a hydraulic motor to feed refuse. He mounted a tank and spray boom on the front of the tractor for disease spore suspensions. Liquid spore suspension causes dry leaf-refuse to stick better to plants.

G. Flowering

Corn has a determinate growth pattern. The vegetative stage (increasing plant size and differentiation) stops at flowering (fertilization), when the reproductive stage (kernel-filling period) begins. Adapted, short-season, temperate corn seldom finishes the kernel-filling period for lack of GDUs because full-season corns are usually grown for higher yields. Under these conditions, earlier-flowering corn plants have smaller plant size and longer kernel-filling periods, while later-flowering corn plants have larger plant size and shorter kernel-filling periods. Plant size and kernel-filling period are negatively associated (see Figure 14.4, Section I E, Section V C).[10,74]

Cross-pollinated species often develop mechanisms to eliminate selfing. Protanderous (male early) flowering served this function in corn varieties. "Silk delay" better describes what happens than "anthesis silk interval." The corn tassel is apical and has dominance. Corn can be under severe moisture stress yet shed a normal amount of pollen at the expected time. In contrast, the female flower is greatly affected by moisture stress as shown both by silk delay and also by number of silks emerged. Silks are 90% water. Silks must be moist to function; therefore, gametophytic selection occurs. All open-pollinated varieties shed pollen before silking. Later varieties have more silk delay because of less natural selection for drought tolerance.[4,74]

Inbreds developed by selfing plants with less silk delay have greater drought tolerance. Hybrid-corn breeders used high-plant-density stress to develop corn with simultaneous flowering. Breeding for stress tolerance (favoring stronger silking) can reduce inbred pollen shed below the amount needed for natural cross pollination. This may be due to smaller tassels or to less auxin, or to both.

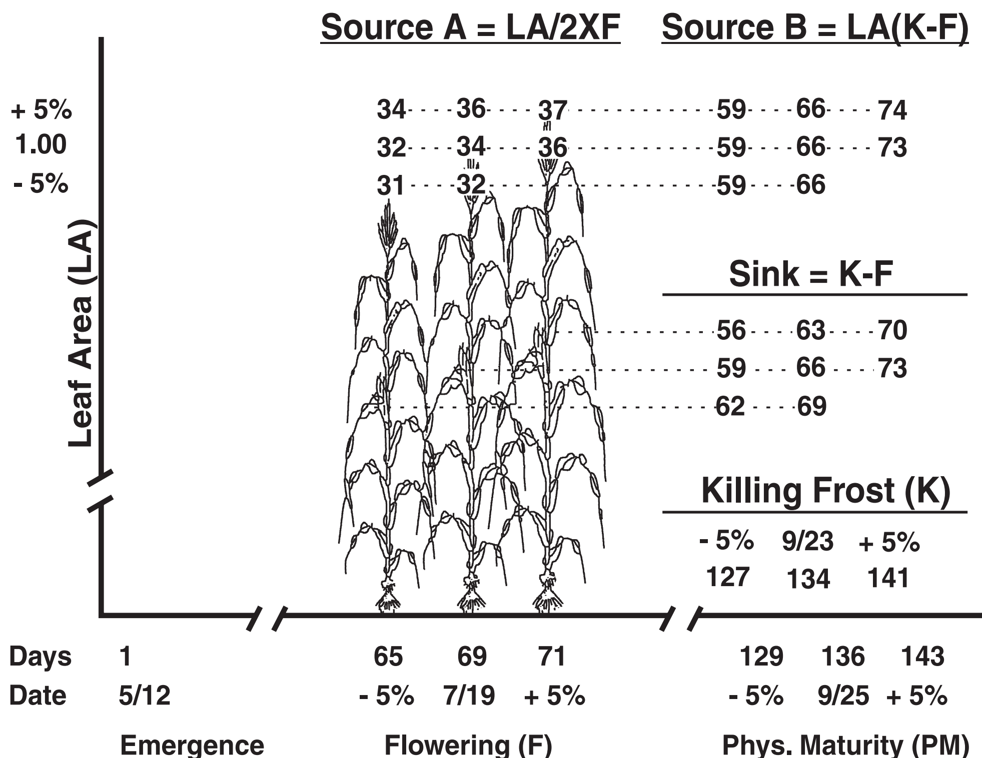


FIGURE 14.4 This figure shows source/sink relationships for early temperate corn relative to flowering date (F), killing frost (K), and physiologic maturity (PM). Flowering date is typically midway between emergence and physiologic maturity. Adapted, early temperate corn seldom reaches physiologic maturity before frost. Later flowering corn is taller (more LA), increasing source. Earlier flowering corn is shorter (less LA), decreasing source A and B, but has more time between flowering and frost increasing sink and source B. Source A increases with later flowering because of more time and more leaf area. Source B values are the same vertically in the matrix because time and leaf area compensate for each other. Sink is a function of time (and rate and stay-green). Both source A and source B can be increased by faster growth before flowering. Sink increases with earlier flowering because of more time before killing frost. As sink increases with earlier flowering, source A decreases by the same amount of time. Source and sink can be simultaneously increased by faster growth and earlier flowering; for example, a corn that grows 5% taller (more LA) than average and flowers 5% sooner than average would have the same source A, 10% more Source B, and 5% more sink than an average corn.

Silk delay (protandery) may be genetic, may be physiological due to poor water uptake or utilization, or may be morphological due to long husks (long silk channel). Selection against silk delay is effective. Silk delay in a female inbred parent makes seed production risky because the best time to plant the male parent is unpredictable. Split male plantings (two dates) are usually used for weak silking females.[4,74]

Shanks are lateral branches connecting the ear to the stalk. Internode pattern of the stalk just above the shank attachment node usually signals the internode pattern of the shank at the ear attachment. Southern dent internode patterns are typically condensed at both places. Inbred Oh43 has a typical Southern dent plant and shank internode pattern that causes tighter husks and slower ear dry down in the field (see Section II B, Section III J).[12]

Shank development reacts to amount of sunlight. Plants growing at a lower plant density, on the border of an alley or on the field's edge, have longer shanks. Shank length is heritable. Long shanks may break during combine harvest or may allow the ear to touch the ground — particularly

on earlier corn (95 RM or less) with lower ear attachment nodes grown in dry seasons. Short shanks may hold the ear upright causing the husks to catch and hold rainfall, causing ear rot. The shank should be long enough for the ear to tip over, but not too long. I use the ear of the plant to judge shank length of selection material. The shank should be no longer than the ear.

Earlier flowering corn yields more than later flowering corn in hot, dry seasons due to subsoil moisture availability during the moisture-critical, stress-susceptible flowering period. Because subsoil moisture decreases over time, earlier flowering corns have more subsoil moisture available when flowering than do later flowering corns.[74]

Silk balling (silks ball-up inside the shoot and emerge late or not at all) is generally limited to inbred B14 derivatives. It is partly a morphological problem associated with keel-shaped husks. The husks and leaves usually have large, prominent midribs. The leaf tips may also be keel-shaped. The keels are concave relative to the cob or stalk. Seed corn companies that use lower plant densities in production fields (because of hand detasseling or because of need for larger kernel sizes) have fewer incidences of silk balling; yet, silk balling does not usually respond directly to plant density — something else is the primary cause. Weather that speeds silk elongation relative to husk development is a factor — probably cloudy, cool, and wet conditions at flowering.[74]

H. Heat and Drought

Heat and drought often occur together because both are more likely in seasons with above-average sunshine and limited cloudiness. Furthermore, soil moisture acts as a buffer to temperature change. So, drought increases the likelihood of heat in the summer. Heat and drought together aggravate and increase damage to corn plants; however, either can be destructive alone. Heat usually damages corn first at the top of the plant, then progresses downward, because younger more succulent tissue is at the top, because less shade is at the top, and because the top is farther from the water source. Corn grown with limited available nitrogen shows leaf desiccation from the bottom up in heat and drought, because mobile nitrogen is transferred from older to younger leaves.[74]

Heat and drought adversely affect corn's female and male flowers. Corn silks are 90% water. Drought often causes physiological silk delay where the tassel sheds pollen at the usual time but silks emerge one to several days later than usual. Corn plants grown at higher plant density show the same response. Silks may desiccate and become nonfunctional because of heat and drought — particularly if pollen is not readily available. Extreme conditions cause tassel blasting that usually occurs when the tassel begins to shed pollen. Amount of pollen may be critically reduced in normal-looking tassels by heat and drought before and during anthesis. Anthers in the lower floret of each spikelet fail to extrude and become trapped in the glumes; in extreme cases, anthers do not extrude.[74]

Heat and drought adversely affect corn leaves by causing wilting and desiccation thus reducing leaf area. The breeder can see a greater horizontal distance in the nursery over time. Desiccation starts with loss of turgor, then follows a progression from leaf rolling to wilting to top firing (heat scald) where tissue is killed. Plants with upright leaves and stiff, erect midribs are more likely to leaf roll (self-shade) than plants with lax leaves. Leaf rolling reduces leaf area exposed to the sun, thus lowering both transpiration and temperature. Plants usually regain turgor overnight, then wilt again later in the day as evapotranspiration exceeds water availability. Wilting and leaf rolling first and most often occur on upper, younger leaves. Top firing usually occurs before flowering on distal portions of young leaves. Genotypes differ in type of leaf necrosis reaction; some show interveinal necrosis, which is a less damaging kind of leaf sacrifice because the form and vascular system of the plant are kept intact.[74]

I. Kernel-Filling Period

After flowering, kernel filling is the final function until the plant dies and loses its green color. Staying green (staying alive) longer is an advantage. Stay-green means long-lived. It means being

healthy late in the season. Stay-green is the opposite of premature death, which is often associated, as cause or effect, with stalk rot. Equally or more important, stay-green increases functional source and sink size. Source is increased because the plant photosynthesizes over a longer time period, later into the fall. Sink is increased because the kernels continue to fill to a later date. Source-sink relationships are particularly important where seasons are limited. Stay-green can be deduced, for individual plant selection, by spray-painting bags with different colors indicating dates when the plant was still alive. Ear rows can be given a 90% dead date by visual estimate. Hybrids in yield trials can be rated for percent green on a given date(s).[18]

Length and rate of kernel-filling period affect yield and test weight in a positive manner. Inbreds may fill rapidly, exhaust their sinks including the stalk, die early, and stalk break in longer, warmer seasons. These same inbreds may stand well and yield well in shorter, cooler seasons. Inbred W117 is an example of this. Higher yielding lines have a combination of longer and faster kernel-filling periods. Stay-green and disease-resistance increase length of filling period. Earlier flowering, when longer days of better quality light occur, higher fertility, available moisture at flowering time, and other factors not yet well understood, speed filling periods. Black layer formation of the kernel usually occurs *after* death of the plant where full-season hybrids are grown. Fully filled kernels have higher test weight.[18]

J. Ear Dry Down

Dry down rate is more important at higher latitudes and at higher elevations where fall days are shorter and cooler (less conducive to drying). Medium- and short-season corn, grown in the central and northern U.S. Corn Belt, is usually dried artificially after harvest. Drying cost relative to new-corn price is important; farmers may delay harvest to allow more ear dry down if corn price is low and drying cost is high. Some farmers avoid drying costs by growing early, fast-drying corns. They market directly from the field and pay the moisture penalty. Grain too dry results in underpayment for dry matter. Late corn, grown in the southern U.S., usually dries in the field before harvest. Hot, humid weather, warm nights, previous stress, and too many tight husks can then increase molds that reduce grain quality.

Fewer, shorter, narrower, looser husks allow more air movement around the ear to aid faster dry down. Narrower, shorter, smaller ears result in more surface area per volume and aid faster ear dry down (see also Section III G).[76] Smaller kernels dry faster in the drier weather.

Full-season corn hybrids seldom complete kernel filling before frost. Drier weather during the grain filling period can reduce yield. Higher temperatures during the fall field drying period can reduce seed germination.[18,74]

K. Stalk Rot

The corn stalk is first used as a sink to store sugars, then as a source to export sugars when grain filling begins. Therefore, a negative correlation between high yield and strong stalks exists. Some breeders increase this stress by removing a leaf or two just after flowering. I prefer using leaf diseases to reduce leaf area. Corn breeders have increased both yield and also stalk quality by selecting for both simultaneously. Higher plant densities and higher nitrogen applications have helped select better stalks. Stalk rot typically occurs in seasons with drought stress at flowering, followed by rains in the fall that provide free water for the pathogen(s).

Brittle stalks (green snap) are stalks that break while still green, usually just before flowering. High wind occurring before or without rain is the causal agent. High wind with saturated soil more likely causes root lodging. Herbicides containing growth regulators may increase susceptibility, but a genetic component exists. Lines from Stiff Stalk Synthetic are more often, but not exclusively, involved. A progression of strong, stronger, strongest, brittle seems to apply. More lignin may be the cause. Once a family of lines is characterized as potentially susceptible, an extra year of testing is in order. Selection screens with mechanical breakage just before flowering may help.

L. Ear Rots

Ullstrup states that corn ear rots have not caused severe losses over extensive areas.[77] However, serious problems have occurred in limited areas on specific hybrids, and it really hurts if one of your hybrids is affected. Heavy rains in the fall increase ear rot problems. Heavy rains in the fall are less likely west of the Mississippi, where poorer grain quality will usually suffice. Problems can occur on short-shanked hybrids that hold the ear erect trapping rainfall at the butt of the ear. Swine can usually identify and reject poisonous feed; however, Gibberella corn ear rot (*Gibberella zea*) is an exception and can be very harmful to swine.[77] Cob rot or structurally weak cobs are usually not a problem in hybrids unless both parent inbreds have weak cobs.

Hybrid ears typically have about 95% of their ovules pollinated. The ear looks fully pollinated until less than 90% of potential kernels develop. The nonpollinated silks persist for a time in the channels between kernel rows. I have wondered if they might contribute to silk-cut kernels. I have not seen enough silk-cut to know.

M. Harvest

Mechanical harvest depends on erect plants and on sufficient ear diameter to prevent ears from passing through snapping rolls. Modern combine heads are very aggressive. You may want to gear down the head for slower, test-plot harvest. If the stalks don't stay erect, they should at least hold together for the gathering chains to pull the plant into the snapping rolls. In nursery harvest, a push, pull, and kick test will help evaluate the stalk for togetherness.

The internode above is noticeably smaller than the internode below the ear attachment node, because the plant starts supporting two active meristems at the attachment node during development. Tops of the plant may break over or break off, usually in late fall. This may be caused by high winds, by European corn borer (*Ostrinia nubilalis*) tunneling, or by stalk rot. Usually this top breakage is late in the fall and does not reduce yield. Topless corn has agronomic advantages. It dries faster because of more air movement around the ears, harvests easier because less trash goes through the combine, and tills easier because stalks need not be chopped. If the corn is topless because of stalk rot, the lower stalks will soon give way (see Section II B).[12]

Our U.S. corn market grades no longer specify kernel moisture, but the former values did have a reason. New corn at 15.5% moisture will keep into winter, at 14% moisture will keep until next spring, and at 11% moisture will keep indefinitely in the U.S. Central Corn Belt. Test weight (kilograms per liter) of corn has negative correlations with moisture content, with cohesion and adhesion of the seed coat, with degree of dent, and with premature death. Kernel shapes that pack better increase test weight. An official test weight cup's height and diameter are equal. Legally, only the 1.057 l (quart size) cup exists in the U.S.

N. Nursery Harvest and Drying

If your nursery is located in or near a seed corn production area, and if your breeding materials are reasonably well adapted, an early frost won't hurt the germination of your breeding materials. Later harvest better evaluates stalk quality. I harvest just in time to make the winter nursery planting dates. I sometimes send high-moisture (not artificially dried) seed to winter nurseries in individual row packets.

Nursery ears in mesh bags usually allow enough air movement that immediate drying is not necessary. In fact, germination of winter nursery corn will probably improve if allowed to dry naturally for 24 hours before artificial drying.

I have always used 41°C (105°F) air for drying nursery seed. It usually takes 3.5 to 4 days for drying — many variables exist. The ears should rattle against each other in the bag, and a dent ear will chatter if twisted when dry enough to store. You can follow drying progress in a drier by checking the air temperature along the airflow. The temperature of the air rises as the corn dries. When it reaches the initial temperature, it has taken out about all the moisture that ambient temperature and relative humidity permit.

Newer driers have better controls and are generally more reliable. I usually sleep next to the drier the first night or two on a cot or on mesh bags. I wake up every hour or so and check temperatures. I have yet to damage corn in a corn drier. Ear quality is more apparent with dry ears. Ear quality will improve over time if you shell your own ears.

O. Interactions

Hybrid-by-year interaction is largest. Growing seasons may be long, short, warm, cool, wet, or dry. Earlier flowering increases grain filling time in shorter or in cooler seasons and usually avoids heat stress in warmer or moisture stress in drier seasons. Stay-green and deeper kernels allow earlier hybrids to increase yield in longer, in warmer, or in wetter seasons.[74]

Different moisture availability at flowering time most frequently causes hybrid-by-location interaction for yield in the central U.S. Corn Belt. Synchronization of corn's imperfect flower

signals better water use. Selecting for stronger silk emergence under higher planting densities (fresh silks are 90% water) allows drought tolerance selection under nondrought conditions.[4,74] Disease and insects can cause interaction, but moderate tolerance is usually enough. Evolution couples higher pest tolerance with lower yield to preserve the species in varying conditions.[17]

Hybrid-by-soil-type interaction is rare. However, in the early 1970s, popular hybrid MO17 × N28 performed very well on older, nonglaciated, hardpan soils (located beyond the Wisconsin glacier) because of inbred N28's exceptional root system that could penetrate hardpans. Soil type affects moisture availability at flowering through its water-holding capacity. Previous crop also affects subsoil moisture; soybeans remove less, sorghum and sunflowers remove more water than corn. Water is the key to most hybrid-by-soil-type interactions in the U.S. Corn Belt.

P. Behavior Summary

Corn is a diploid, cross-pollinated, monecious species with an imperfect flower. It is easy to pollinate. Corn is well designed for sunlight interception and has efficient C-4 photosynthesis. Plant your nursery early to select better-growing inbreds under cool conditions. This helps develop an efficient canopy early in the season when longer days of more direct sunlight occur. A population segregating for flowering date shows the earlier flowering plants being taller in the first half and the later flowering plants being taller in the last half of the flowering period. Early corn grows faster for a shorter period. Late corn grows slower for a longer period. Earlier flowering corn has smaller final plant size because of the determinate growth pattern. Plant size and kernel-filling period are negatively associated. Later flowering, taller plants have shorter filling periods. Silk delay signals poor stress tolerance. Hot, dry weather most often reduces yield. Upright leaves often signal downright roots. Adapted, early, temperate corn seldom completes kernel filling for lack of GDUs. Ear dry down rate is more important where shorter, cooler days occur in the fall.

IV. BREEDING SUPERIOR INBREDS

A. Phenotypic Selection During Inbreeding

One must know what traits to select to accomplish effective phenotypic selection during inbreeding. Useful heritable traits include good germination in both warm and cold conditions, vigorous seedling and juvenile plant growth, proper flowering time that also affects plant height, ear height, and kernel moisture, and strong flowering (both silking and pollen shedding). Additional useful factors include leaf disease tolerance, heat and drought tolerance, stalk rot tolerance, stay-green, kernel texture (degree of dent or amount of hard starch), and factors affecting ease of harvest, such as loose husks and ease of shelling.

Select the earliest flowering plants by segregating populations to capture vigor genes is best. Then pollinate only the earliest 10% of S1 or BC1 populations. I usually have about 0.4 ha (1 acre) of BC1 populations at 162,000 plants per hectare (65,000 per acre). I have 20 to 30 populations of 2000 to 3000 plants per population, usually make 6500 pollinations, and save 1500 to 1800 ears. I rarely select ears with less than 14 kernel rows for the U.S. Corn Belt or with less than 12 for Northern Europe. Intermediate kernel texture is generally selected. The most useful trait for me has been tolerance to high-plant-density (HPD) stress, which tends to eliminate plants with delayed or weak silking before pollination. HPD stress also causes increased barrenness and scatter-grain ears as a basis for discard at harvest.[4,12] It is efficient to select for traits associated with yield under stress that have higher heritability than yield itself.[78] I artificially inoculate with indigenous leaf diseases and select for stay-green. To determine stay green it is useful to identify, every week or 10 days in the fall, bags on plants that are still alive. This can be done with different colored spray paint.[18]

I select on a row basis at S2 and beyond and send 1800 S2 ear rows to winter nurseries. These are planted at twice normal plant density. I discard 50% before flowering based on cold test germination, seedling vigor, juvenile plant growth, and uniformity within row. Discard rows are flagged off with shoot bags. I shoot bag four and pollinate three plants per row (see Section IV D). I pollinate four on better rows and even more on the best growing rows where I have shoot bagged extra plants. Saving as many ears as were sent simplifies planning. I only save ears from rows with two or more good ears. But, I save more than two ears on better rows. On some rows I only save one ear.

I grow S3s in three environments in summer nurseries — early and late planting at the home nursery, and at an additional, convenient location. Fifty percent of the rows are discarded based on cold test germination, on two sets of seedling vigor and juvenile plant growth scores at each of the three plantings, and on uniformity within rows. Rows are chosen for strong germination and vigorous, uniform growth. I pollinate, select, and harvest, usually at the home nursery first planting, as I did at S2. I harvest as late as possible while still being able to prepare for winter nurseries.

I cross S4 seed of S3 families to testers. I bulk only enough S4 seed from selected S3 plants from each selected S3 family (row) to test cross; on average about 50 S3 families per selection pedigree. If 25 selection pedigrees survive, that is 1250 different S3 families. If appropriate testers are available in winter, I cross each S3 family to two testers for 2500 hybrids to be grown at four or five one-replication locations. In practice some of the crosses are made the next summer in isolations or in paired rows, and some S3 families will be discarded. I rarely expand an S3 family for test crossing. I decrease number of families for selection over generations of selfing.

I grow S4s and beyond ear-to-row at normal plant densities because I am more interested in seed increase than in additional selection at HPD. I bulk seed of sister rows that appear identical at S5 or beyond. Each new inbred usually represents one S3-row family.

The aforementioned traits (first paragraph) are useful in inbred parents and necessary in commercial hybrids. For commercial single crosses, each inbred parent should be average or better in hybrids for all important quantitatively inherited performance traits. Phenotypic selection during inbreeding is useful. Doubled haploid schemes that lack selection during inbreeding are rarely used in corn.

Developing inbreds that have superior performance in production fields will help your company have a reliable seed supply for the farmer. Uniform, true breeding inbreds that can be identified for certification inspection and meet uniformity standards are necessary. Female inbred *per se* yield should be at least average with a small standard deviation. Disease tolerance should be at least moderate. Female inbred seed yield should be at least 90% saleable; i.e., not more than 10% discarded for seed size, poor germination, etc. Warm germination should average 95% or more, cold germination 80% or more, and have shelf life of average or better. Female inbred should be non-tillering, easy husking, and have average or less field shelling loss during harvest. It should harvest easily and cleanly. Tassel extension (peduncle length) should be average or longer for mechanical detasseling.

Same-time planting of male and female inbreds in the production field is desirable. *It helps*. Female planting delays are usually unacceptable because they reduce seed yield and reduce seed quality. Females that are up to 70 GDUs earlier can be planted the same time as the male with extra isolation. The maximum male delay is about 150 GDUs. The longer the delay the more likely the male seedbed will need new tillage preparation. Female inbreds susceptible to silk delay require a split planting (two dates) for the male.

B. Test Crossing, When and What For

As stated earlier, correlations between inbreds and their hybrids are imperfect. Test crosses are necessary to determine hybrid performance. New inbreds may be test-crossed in early stages of development (S0 to S2) or may be test-crossed in later stages of development (S3 or later).[65] Bauman surveyed U.S. breeders to show 18% test cross at S2, 33% test cross at S3, 27% test cross at S4, 9% test cross at S5, and 9% test cross inbreds at later than S5.[79] Advocates of early testing believe that

high yield is so important and so elusive that it must be identified early and carefully followed during inbred development. They generally have less concern for performance *per se* in seed production fields.

Advocates of late testing believe yield potential is changing due to genetic segregation during inbred development. Bernardo reports the genetic correlation between test cross performance of inbreds and their directly descended homozygous inbreds. They are 0.71 for S1, 0.87 for S2, 0.93 for S3, 0.97 for S4, 0.98 for S5, and 0.99 for S6 inbreds.[80] Later stages of inbred development, when more loci are fixed, provide more accurate testing. Bernardo determined that a greater number of inbreds need to be retained with early testing.[81] Advocates of late testing believe more useful variation (opportunity) for selection exists at later stages, and more phenotypic selection during inbred development helps seed production.[82] Missouri and Iowa State Universities have developed elite inbreds via early testing.

Availability of funds affects choice of either early or late testing, because early testing is more expensive. More poor inbreds are tested with early testing because many poor inbreds can be discarded for *per se* performance during inbred development. Making crosses and yield testing for early testing are each more expensive than selfing with selection for later testing. However, winter isolated crossing blocks, where the male inbred is the tester and the tested inbreds are detasseled females, reduces time and cost of making test crosses.

Jensen et al. gathered extensive data from six experiments at three locations for 2 years.[83] They correlated yields of S2s and S5s both *per se* and also with tester. All generations were grown in the same experiment in the same years to minimize environmental interactions. Not surprisingly, S2 test cross yield correlated higher with S5 test cross yield than did S2 *per se* yield with S5 test cross yield. Also S2 *per se* yield correlated higher with S5 *per se* yield than did S2 or S5 test cross yield with S5 *per se* yield. What you test is what you can predict. Their experiment was designed primarily to evaluate S2 *per se* yield testing for predicting S5 test cross performance. S2 *per se* yield very poorly predicted S5 test cross yield — only 2 of 12 correlations were significant (0.40* and 0.43*). S2 test cross was better, but still poor. The 12 phenotypic correlations between S2 test cross and S5 test cross only averaged 0.63* for yield.[84] Variation among S2 test cross yields explains only 40% of the variation among S5 test cross yields. Among eight agronomic traits, the highest average correlation between S2 test cross and S5 test cross was 0.71** for stay-green.[84] The correlations would be poorer yet when S2 test crosses are grown in different years, in different locations, and in different fields than S5 test crosses. I see their results as evidence for test crossing at a later stage than S2 to predict S5 test cross performance.

Using their test cross protocol as if it were a breeding method, would a better inbred be identified by eliminating the S2 yield testing and doubling the number of S5 test crosses (from 132 to 264 entries)? That is the question. Yield-test expense limits the number of new inbreds. I prefer a greater number of new inbreds test crossed at a later, more accurate stage of inbreeding.

Weyhrich et al. made direct comparisons of response and of efficiency for four or more cycles of seven methods of recurrent selection in Iowa BS11. This study provides interesting, useful, corn breeding information — including cost.[68]

You have a limited amount of time and money. Time to become productive is also limited. You will not succeed by chance alone but, instead by better planning and by better execution than your competition. A time and motion study will help you use both time and money more effectively. What did Jensen et al. do with the materials between S2 and S5? Where and how were the materials further evaluated and selected?[83] What phenotypic and natural selection occurred? Should or could wide area testing happen sooner? You will need to fit your inbred development and testing philosophy into a system that fits your available facilities.

I like to test cross S4 seed representing an S3 row, because I see the S3 rows in three environments — I am confident about my selections. If I make the test crosses in the winter, I can see their single

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* Significant at P ð 0.05.

** Significant at P ð 0.01.

crosses the next summer (fast feedback). I tried test crossing BC1S1 (S2) ears in the winter, but missed not having a progeny test (row) for phenotypic selection before test crossing. Test crossing S3 bulked seed representing an S2 row is a little better, but I grow S2s in the winter usually in only one, atypical environment for phenotypic selection. I am not as confident about those selections.

I have developed or co-developed four inbreds that were female parents of easy to produce (very profitable), very popular hybrids (3732, 3737, DK250, and DK535). I believe late testing and phenotypic selection helped. Certainly, their ease of production increased their sales. Foundation seed of popular hybrids is often limiting. Ease of production probably also improved their hybrid performance. Hard to produce hybrids usually show it, e.g., a female that brings husks and shanks into the drier dries poorly and the hybrid germinates poorly.

Early testing is a way to start. Everyone likes to have test cross data. It gives you an immediate, personal (selfish) interest in your company's testing program. That's healthy. As you get more experience and more confidence, you will probably do more late testing. Go to the field, study the materials. What does the corn need to perform better? Think about it, then go back and study the corn some more. How can you better adapt the corn to its environment? Some good objectives that require phenotypic selection and later testing will surely occur to you.

Testers with a broad genetic background, such as synthetics or open pollinated varieties, test for general combining ability or additive gene action. Testers with a narrow genetic background, such as inbreds, test for specific combining ability and for whatever type of gene action that occurs in that particular cross. Commercial use of single-cross hybrids has rightfully increased interest in specific combining ability, but GCA is as important as ever.

It is convenient to place families of related materials into heterotic groups and to place hybrids among these groups into heterotic patterns. Heterotic patterns are determined empirically by relating the heterosis in crosses to the origin of the parents in the crosses. Heterotic groups are closely related materials that react in a similar manner in heterotic patterns. Such parentage (heterotic groups) can be varieties, synthetics, or inbreds. Narrow classification for grouping (inbreds) increases efficiency because fewer crosses are necessary to find the best hybrid. Heterotic groups and patterns are heritable; new inbreds are likely to be similar to their parents. Some useful inbred heterotic patterns are listed in Table 14.6. Again, I prefer heterotic group classification based on inbreds. Fewer heterotic groups simplify decisions, but severely limit progress. If Stiff Stalk Synthetic by Lancaster Sure Crop were the only heterotic group, the last four most popular hybrids (3732, 3475, 3377, and 3394) would not have occurred. None of them contained Lancaster Sure Crop variety germplasm.

C. Nurseries, Locations and Organization

Most corn research organizations in the temperate area have both summer and winter nurseries where their materials are grown and pollinations are made. Winter nurseries are grown at some accessible place where successful winter crops occur and the materials can be returned in time for the summer yield testing and nursery planting. Weather data on some winter nursery locations are listed in Table 14.7.

Usually, summer nurseries are grown near home or near office to reduce travel, but some corn breeders prefer separate disease (natural or induced) nurseries that may be located where it is easier to cause the disease. Ear rows may be evaluated for disease or for other traits at a distant location and be pollinated at the home nursery if selection is on a row basis. I usually grow ear rows for phenotypic selection at two environments in addition to the one where they are selfed. Multiple observations help develop inbreds that perform better in more environments, including in seed production fields.

Efficient corn breeding programs use time particularly well during the pollinating period. The nursery is usually divided into blocks or fields by kinds of pollination. The selfing block is all selfing, the crossing block is all crossing, etc. Areas that involve sibbing, backcrossing, or selection

TABLE 14.6
Some Useful Heterotic Patterns

Inbred 1	Inbred 2	RM	Inbred 1 Family	Inbred 2 Family
A509	x IDt's early	90	NWest dent/Minn. #13	Iodent
A619	x A632	102	Lancaster/Minn. #13	Stiff Stalk (B14)
A632	x B37's early	105	Stiff Stalk (B14)	Stiff Stalk (B37)
A632's early	x F2	85	Stiff Stalk (B14)	Lacaune flint
A634	x Mo17	105	Stiff Stalk (B14)	Lancaster/Krug
A634	x W153R	100	Stiff Stalk (B14)	US133 (Minn. #13)
B14	x Oh43	115	Stiff Stalk	Lancaster/Minn. #13
B37	x B73	112	Stiff Stalk	Stiff Stalk
B37	x C103	118	Stiff Stalk	Lancaster
B37's early	x Idt's early	105	Stiff Stalk (B37)	Iodent
B37	x Idt's late	115	Stiff Stalk	Iodent
B37	x Oh43	115	Stiff Stalk	Lancaster/Minn. #13
B73	x C103	117	Stiff Stalk	Lancaster
B73's early	x CO255	100	Stiff Stalk (B73)	Inra 258, four way
B73	x Idt	105	Stiff Stalk	Iodent
B73	x Mo17	115	Stiff Stalk	Lancaster/Krug
B73	x Oh07	120	Stiff Stalk	Learning
B73	x Oh43	111	Stiff Stalk	Lancaster/Minn. #13
C103	x Oh43	112	Lancaster	Lancaster/Minn. #13
C103	x WF9	118	Lancaster	Reid
C103's early	x WF9's early	105	Lancaster (C103)	Reid (WF9)
CM105	x F2	80	Stiff Stalk (B14)	Lacaune flint
CM105	x CO255	80	Stiff Stalk (B14)	Inra 258, four way
CM7	x F2	75	Ottawa flint/Mixed dent	Lacaune flint
CO109	x WF9's early	90	Early Butler	Reid (WF9)
EP1.F7	x F115.W33	80	Euro. flint	I153; Golden Glow, Minn. #13
F2	x Idt's early	85	Lacaune flint	Iodent
F2	x Mo17's early	90	Lacaune flint	Lancaster/Krug
F2.F7	x W401	82	Lacaune flint	Minn. #13/Golden Glow
Idt's late	x WF9's late	120	Iodent	Reid (WF9)
Oh43	x W64A	110	Lancaster/Minn. #13	Reid (WF9)/Krug
W117	x W64A	95	Minn. #13	Reid (WF9)/Krug

then crossing may also be separate. The idea is to do the same type of operation at a particular place, to do it more routinely (quickly), and to minimize mistakes.

Rows may be identified by strips of heavy paper stapled around the end plant of the row. Both the paper and the ink should be weatherproof. Usually a range and row number grid system is used that also indicates relative coordinates of the block or field. If the nursery is very large, a block number may also be needed on the tag. Color of the tag may indicate number of pollinations expected or instructions of some other kind. The crossing block is made up of paired rows where materials to be crossed are grown in adjacent rows. Tag color may indicate pairs. If the rows to be crossed differ for maturity, the earlier row is planted later based on GDUs to flower.

D. Pollination, How and Why

Corn is a hermaphrodite. It is easy to pollinate because its male and female flowers are separate, making it easy to cross plants, yet both flowers are on the same plant, making it easy to self-pollinate (see Section III G, Table 14.5). We provide instructions on index cards as a reference for pollinators in the beginning of the pollinating season. The idea is to transfer pollen from the tassel

TABLE 14.7
Daylength for December 22 and Mean December Temperature (°C) for Potential Winter Nursery Sites

Area	Latitude (degrees)	Daylength December 22 (h)	Daylength December 22 (min)	Temp.[a] December (mean °C)	Chance of Good Crop	Main Problems
Brownsville, Texas	26°N	10	29	23	Good	Frost
Homestead, Florida	25°N	10	33	26	Very Good	Frost
Havana, Cuba	23°N	10	42	27	Good	Politics
Maui, Hawaii	21°N	10	51	27	Very Good	Storms, labor
Puerto Vallarta, Mexico	20°N	10	55	29	Good	Politics
San Juan, Puerto Rico	18°N	11	03	27	Poor	Insects, labor
Ho Chi Minh City, Vietnam	10°N	11	32	28	New	New
Lusaka, Zambia	15°S	13	01	26	Very Good	Distance
Santiago, Chile	34°S	14	26	19	Good	Slow, cool
Buenos Aires, Argentina	35°S	14	31	22	Good	Inflation
Cape Town, South Africa	35°S	14	31	19	Excellent	Slow, cool
Aukland, New Zealand	37°S	14	51	20	Excellent	Slow, cool

[a] Weather records courtesy of Dr. Wayne M. Wendland, State Climatologist, Illinois State Water Survey, Urbana, IL 61801.

of a plant to the silks of the same plant to self-pollinate and to transfer pollen to silks of another plant to cross-pollinate. If the crossed plants are related, the cross is called a sib.

Pollinating is a repetitive, routine operation. Bags are usually put up one day and taken down the next to ensure that pollen in the bag is from the bagged tassel. Bags can be put up using a stapler to fasten them, but paper clips are much easier and faster to remove when taking bags down. Taking bags down is done in the critical, limited, pollen-shedding period when it is most important to be efficient. One should eliminate unnecessary steps or movements during pollinating and plan ahead by visually locating the next plant to be pollinated while pollinating the present plant. Effectiveness of a pollination depends both on collection and also on dispersal of the pollen; each of these operations is improved by bag movement — better pollinators rattle or rustle the bags more than poorer pollinators when taking bags down. Pollinating bags have pleats; pollen dispersal on silks is greatly enhanced by pumping the bag like a bellows (holding the bag by opposite folds) as the pollen is dumped on the silks. Each seed is dependent on at least one pollen grain on each silk. Nursery books wear better in your apron at pollinating time if protected in a pollinating bag during damp or wet weather.

PEPPER is an acronym for pollinating end plants planted at extreme rates (shoot bag four, pollinate three plants). As we increased plant densities in our selection nurseries, the number of shoot bags used increased proportionally, even though the number of pollinations was held constant. This became a serious problem when planning labor and supplies for the pollinating period. We needed a simple, effective method to limit shoot bagging. As I studied the selection practices of more experienced breeders, and as I became more experienced myself, I found stronger arguments for selection among rows rather than selection among plants within rows. I also experienced some extreme heat and drought seasons when it was difficult to pollinate plants in the center of selection rows planted at high plant density. Putting the three concepts together; the need to limit shoot bagging, preference for row selection, and the need for easier pollinating under heat and drought conditions resulted in PEPPER. Most corn breeders, after thinking about it and trying it, use PEPPER.

E. Materials, Choice of Germplasm

Superior new inbreds come from the best existing inbreds, as would be expected from recurrent selection theory.[85] Breeding starts should be chosen based on research data because facts are better

than opinions. A good hybrid testing program is essential to recognize the best inbreds and to identify their relative strengths and weaknesses. The testing program should have two objectives. It should quickly identify marketable hybrids and it should quickly identify the best inbreds for new breeding starts. Because genetic gain is being made, good inbreds have a time value like interest on money — time lost is never regained (see Section V C).

General combining ability (GCA) is very important. It results from cumulative, additive gene effects that help the inbred perform well in multiple environments. Of course, GCA effects also help the inbred perform well in multiple crosses, because each tester is a genomic environment. On several occasions I have seen the best hybrid for a geographic area result from crossing the two best (GCA) nonrelated inbreds for that area. I have also seen the opposite; i.e., flash-in-the-pan, short-lived hybrids that had parent inbreds with high specific combining ability under certain conditions and with only average GCA. Additive genetic effects lend themselves to successful cyclic selection better than nonadditive effects.

It is very important to determine relative value of inbreds (GCA) based on an objective sampling. Each inbred's crosses should be in the database due to the same criteria to avoid bias. General combining ability should be based on the average of as many single crosses as possible. At first, the top cross or an open pollinated variety was the traditional way to compare GCA for inbreds. Diallel crosses became popular during the double-cross era, because male and female single cross parent yields were important, and also because diallel crosses insured availability of all parent seed needed to make any predicted double cross hybrid.

Balanced sets or matrix tests (also called partial diallels) became popular for GCA with the commercial single-cross-hybrid era. Each inbred is crossed on five or six tester inbreds for GCA evaluation. Some prefer the same tester inbreds for all tested inbreds. Others prefer an alternate inbred tester where a relationship between tested and tester occurs. Still others prefer different inbred testers for each family of tested inbreds. In the last case, valid comparisons are made only within tested inbred families. By contrast, modern methods to determine GCA involve hundreds and even thousands of tests for a particular inbred. To obtain critical information on barrenness, root lodging, brittle stalk breakage, and dropped ears, rare discerning environments must be sampled or special tests devised.

Tens of thousands of new inbreds are developed and tested each year. Thousands are good enough the first year to warrant testing a second year. Hundreds of inbreds are used in commercial hybrids, but only a few are used extensively. Perhaps 5 to 20 elite corn inbreds exist in the world at a given time. Fewer elite inbreds exist when assessed over longer time periods. The development of an elite inbred is an extremely rare event. Your choice of recurrent parents (3/4 or 7/8) for breeding starts should be limited to elite inbreds in popular commercial hybrids and to inbreds that appear to be becoming elite.

Choose the best elite inbreds for your area to use in breeding starts. Study your personal testing program. Which elite inbreds are successful in your area of responsibility? Which ones fail? Why? Can you fix or reduce their limitations for your area through breeding? Study the results of your company's testing program. Which inbreds are in precommercial, or in pilot-level hybrids? Study outside testing programs. Is any new inbred from federal, state, or foundation seed programs successful in two or more hybrids? A good breeding strategy is to identify and to correct whatever prevents an elite inbred from being better.

Each elite inbred contains a combination of genetic traits that satisfies the market place. Elite inbreds are so important to the seed corn industry that one should utilize them as effectively as possible in breeding starts. I usually backcross once or twice and sometimes even more times to the elite inbred to obtain breeding populations with a very high percentage of the elite inbred before starting selection. I call this genome conservation because I want to preserve (conserve) as much of the elite genome in the new inbred being developed as possible. The odds against developing a new elite inbred are about a million to one. The long odds are probably due to epistasis caused by interlocus interaction among chromosomes. Preserving as much as possible of the elite genome in

the new inbred is logical. Use your breeding skill to choose the best nonrecurrent sources and to develop effective screens for phenotypic selection during inbreeding.

The highest possible performance mean (for yield and for yield/moisture) in a breeding population is your most important priority. It is much more important than having a lot of variation for selection at a lower performance mean. I often use related inbreds as parents of breeding starts, then backcross to the better (yield and yield/moisture) inbred. I try to improve an elite inbred with the best possible related inbred. For example, improve inbred B37 with another B37, or improve an elite Iodent inbred with a sister or a parent of that inbred. Of course, wider crosses are sometimes necessary to obtain the trait(s) needed for improvement. Two backcrosses should be the minimum in that case with selection for the new trait while growing each backcross generation if possible. The emphasis should be to keep what you have that is both rare and good. Where interlocus epistasis among chromosomes is important, you need nearly all of the elite, original genome. For the same reason, I avoid sibbing elite materials because it scrambles favorable linkages.

Specific combining ability is also important. Nonadditive gene action helps sort out the best single-cross combination between two groups of closely related inbreds. Epistasis may account only for the last 3 or 4% of heterosis, but those last few percentage points are often the difference between a winning and a losing hybrid.

Genetic diversity is important. Genome conservation and increased genetic diversity for a geographic area can be achieved simultaneously. The Rinke method makes a late elite inbred earlier so it can be used in a shorter-season area, thus increasing genetic diversity in that area. Minnesota inbred A632 is an earlier form of Iowa inbred B14. A632 was very popular for more than a decade in the northern U.S. Corn Belt.[32] The Early Iodent inbreds were developed by the Rinke method. They have remained popular in the northern U.S. Corn Belt since the 1970s.[12] Both A632 and the Early Iodent inbreds also became popular in Europe and in other areas where 100 to 105 RM corn is grown. These inbreds effectively increased genetic diversity in shorter-season areas. They are examples of moving a larger percentage of dent germplasm to higher numbered latitudes.[12] Making corn earlier is a natural thing to do from a corn evolution standpoint.[12]

F. Breeding Methods, Which Ones and Why

The most often used plant breeding method in corn is the pedigree method. It emphasizes knowing the materials thoroughly, choosing parents with complementary traits for breeding starts, and keeping detailed progeny records that show family relationships. The pedigree method uses progeny tests from ear-to-row plantings where the progeny in the row show the worth of the parent ear and where the progeny rows of the sister ears show the worth of the grandparent ear. These ear rows can be grown in multiple environments to better determine degree of heritability. Specific strengths and weaknesses tend to be associated with inbred families. FD Richey described the cyclic use of the pedigree method, calling it cumulative selection.[46] He predicted that over time, cumulative selection would reduce the number of deleterious recessive genes in corn inbreds, thus improving inbred–hybrid correlations for yield. Today's inbreds are much improved for *per se* performance over the first ones, so perhaps he was right.

The backcross method is used to transfer a simply inherited trait to an elite inbred. The source of the trait is called the donor or nonrecurrent parent, and the elite inbred is called the recurrent parent. Each backcross generation has a complete genome from the recurrent parent inbred in each plant coupled with a mixture of the recurrent and nonrecurrent genomes for the other half. Segregation for remaining heterozygous loci starts the generation following the first self when maximum opportunity for selection occurs. In some cases (e.g., waxy), special tests must be made to identify presence of recessive genes, and in other cases (e.g., corn borer and high amylose), modifiers are so important for a trait that alternate selfing and backcrossing generations are necessary to get the expression of the desired trait. Backcross populations are useful for pedigree selection. The backcross one (BC1) is an excellent generation for

identification and selection of desired highly heritable traits, because the complete recurrent parent genome in each plant acts as a tester for the desired trait from the donor parent genome.

Many elaborate breeding schemes have been described for corn improvement, because corn is so easy to cross and to self. Some methods emphasize what to select. Some methods seem complex at first because they involve test crossing and hybrid performance evaluation while developing inbreds. To my knowledge all methods use the pedigree method for final development of the inbred — even monoploid and somoclonal variation methods. Thorough knowledge of the pedigree method will be most useful to you. It will also help you understand more complicated schemes.

Population improvement at Iowa State University has been especially effective. A 50-year program of recurrent selection for general and for specific combining ability on Stiff Stalk Synthetic has developed three elite inbreds (B14, B37, and B73; see Section II E).[49] These are very costly (time and money) forms of recurrent selection.[68] I give much credit to the excellent people — staff and students (people make things happen), to their very good testing program, to their very good facilities, and to their prolonged continuity and perseverance for the excellent inbreds developed.

Simple, phenotypic recurrent selection for early flowering provides a cycle of recurrent selection per generation. Late materials can respond one day, and adapted materials one-half day earlier to flower per cycle of selection.[67,86] One synthetic that I selected by this method is half of a parent inbred of a very popular hybrid (3751). The synthetic contributed early flowering and stress tolerance.[4] Any trait that can be evaluated before flowering (ear height, early flowering, HPD stress, leaf blight, prolificacy, rind puncture, spring vigor, etc.) lends itself to this type of recurrent selection. It is economical and effective for highly heritable traits.[68] I use simple, phenotypic recurrent selection (population improvement) to develop nonrecurrent parents (1/4 or 1/8 of the population) for pedigree selection.

Choice of breeding method should be determined by the breeding objective, by the materials available, and by the money available. Experienced breeders do more selfing and less sibbing, and they also do more row selection and less individual plant selection than their younger colleagues. Early testing quickly expands hybrid yield testing, which in turn expands yield test results, expands data processing, and expands decision making. Early testing requires more yield test activity per useful inbred developed than late testing.[81] Competitive position affects choice of method. Are you trying to catch up, stay even, or get further ahead? Falling behind increases desperation and the need for more apparent activity (see Section V J). Early testing will increase apparent activity. Winter facilities and winter isolations available also affects method choice. Early testing requires more crossing space to make hybrids during the winter. Late testing requires more selfing space for advancing inbred development during winter. Again, objectives, materials, and dollars should be the main factors in choosing methods.

G. Developing Inbreds, Who and How To

Cumulative selection, cyclic pedigree method with late testing, is an excellent way to develop inbreds.[46] With modern winter nurseries, modern plot equipment, modern data processing, etc., recurrent selection with late testing is nearly as fast per cycle as recurrent selection with early testing. And, usable inbreds are available at the same time as hybrid yield test results. My version of cumulative selection is called the FAST method. It attempts to coordinate inbred development and hybrid testing with various other requirements for commercial production (Table 14.8). The 4-year plan was used to develop 3732, DK672, and other commercial hybrids.[12,55]

Benjamin Duddleston studied plant characters affecting yield in Reid Yellow Dent at Iowa State.[87] He grew several experiments involving individual plants, ear rows, human judges, and progeny yield tests over a two year period.[87] Based on his results, he would select medium-large to large healthy, erect stalks with drooping (not upright) ears at medium to high ear height. He would select plants without tillers and avoid extremes in tassel morphology. He would select mature, sound, medium-sized ears with well-filled butts, 14 to 20 kernel rows and medium-wide space

TABLE 14.8
Fast Method — Two Plans

Year	Instructions
	4-Year Plan
1	Grow elite by elite inbred line S1's or BC1's under HPD stress; self and select.
1W	Grow S2's during winter and self. Discard rows that grow poorly, are disease susceptible, etc.
2	Grow S3's under HPD in three (or more) environments; use PEPPER. Make single crosses in isolation of S3 lines on to two or three best inbed lines (based on SCA); avoid difficult-to-produce hybrids.
2W	Grow two or three ears of each of the superior S4's ear-to-row to self.
3	Grow single crosses in a two (or more) location test. Grow S5's ear-to-row and self; bulk a single row if family is uniform among and within rows (if not uniform, use 5-Year Plan). Remake single crosses with S5 bulk seed.
3W	Remake better single crosses with S6 bulk seed if more seed is needed. Increase surviving inbreds.
4	Grow Single crosses in many tests over a wide area. Increase surviving inbred lines. Grow inbred lines in flowering tests and disease tests.
4W	Foundation seed makes 50 to 100 bushels of each superior hybrid and increases needed parent(s).
5	Grow superior hybrids to strip plots. Grow parents in flowering, tests, disease tests, and parent tests. Produce two 32 ha (80 acre) fields.
	5-Year Plan
1 thru 3	Same as above
3W	Nothing has to happen. Can grow S6's ear-to-row to self. Can remake single crosses.
4	Grow surviving single crosses in an advanced test. Remake surviving single crosses with S6 bulk seed. Increased surviving inbred lines; bulk if uniform among and within rows.
5	Grow surviving single crosses in many tests over a wide area. Increase surviving inbred lines. Grow new inbred lines in flowering tests and disease tests.
5W	Foundation seed makes 50 to 100 bushels of each superior hybrid and increases needed parent(s).
6	Grow superior hybrids in strip plots. Grow parents in flowering tests, disease tests, and parent tests. Produce two 32 ha (80 acre) fields.

between kernel rows. He would prefer ears with plumper, deeper, heavier kernels.[87] He applied these selection criteria at Purdue University to develop inbreds 461-3, 66, B2, Fe, Tr, and WF9.[12]

Raymond Baker, of Des Moines, IA, often used a modified backcross approach to make elite, late inbreds earlier. He grew large (>1000 plants) F2 populations from early by late inbreds and selfed the earlier flowering plants. At harvest he would save ears from plants that had desired traits of the late, elite inbred. He would grow these S2 ears ear-to-row and verify both the earliness and also the presence of desired traits from the late inbred before backcrossing and repeating the procedure. He sometimes went to S3 rows to verify desired traits before backcrossing.

Perry Collins, of Algona, IA, developed many useful inbreds and popular hybrids during the double-cross hybrid era. He took heterotic patterns one step further (epistasis). He judged inbred performance in double-cross hybrids. His breeding starts improved the weakest inbred in a superior double-cross hybrid. He evaluated the improved inbreds directly in the double cross. It required, and he had, an excellent testing program. My first two commercial hybrids (3670 and 3676) substituted a leaf-blight resistant inbred for the weakest inbred in two of his popular, double-cross hybrids.

Dr. Rinke, at the University of Minnesota, selected for early flowering in large backcross populations to develop earlier forms of elite, late inbreds.[32] His method is used in the northern U.S. Corn Belt and in northern Europe.[32] He used inbreds that differ in flowering time by two weeks

or more. He used large one-backcross and two-backcross populations (>500 plants) grown at twice normal plant density to select for earlier flowering among predominantly late genotypes. Results using Iowa inbred B14 and Indiana inbred WF9 showed recovered inbreds flowering 5 to 10 days earlier than their recurrent parent (see II E6). In hybrids, the recoveries averaged 13% more yield and 4% less stalk breakage than comparable-maturity recommended check hybrids.[32] The Rinke method effectively selects for vigorous growth because vigor genes help cause earlier flowering.

I have used variations of the Rinke method for 40 years. Raymond Baker told me the most common problems in making late, elite inbreds earlier are poor performance of the earlier form of the late inbred in the shorter-season area, and slow drying. To reduce the likelihood of these pitfalls, we prescreened late, elite inbreds in single cross trials using very early inbreds as testers. The idea was to evaluate hybrids of the better late inbreds in the short-season area before doing all the breeding. The very early testers served to cause flowering of the hybrids at the proper time for the shorter-season area. We did this on a regular basis for about 20 years and it was useful.

Dr. Bob Rosenbrook, who worked with me at Mankato, suggested we could better evaluate individual plant segregates if the ears were selfed rather than backcrossed in summer. This allowed normal planting date and better pollinated ears that unmask lack of stalk quality and lack of stay-green. We selfed in summer and backcrossed or sibbed in winter. This was useful. It helped phenotypic selection in summer, and it helped making crosses in winter where days are shorter and flowering time is usually compressed. I have developed several useful inbreds for later hybrids by failing to make the late inbreds much earlier, but they were improved in some other way (usually more stress tolerant).

Dr. John Hoffbeck of Tipton, IN has many useful ideas. He has used HPD (more than twice normal) extensively for phenotypic selection in inbred development and in hybrid selection. After seeing Dr Hoffbeck's narrow nursery alleys (45 cm), Mr. Baker adjusted his nursery planter for zero alley width. John believes strongly in phenotypic selection: "I believe, by choosing only the very best appearing materials when grown under stress, we can greatly reduce the number of failures and thereby markedly reduce the cost per useful inbred developed." John starts selection with only the best (GCA) pedigrees, and culls pedigrees each advanced generation of selection. He discards ruthlessly. His testing program (central Indiana to central Michigan) was oriented north-to-south for all tested hybrids. John studied each hybrid during shorter cooler, medium length warm, and longer hotter seasons each year: "All new hybrids must be widely adapted. Too many hybrids result from the coincidence of random errors and interactions that mistakenly indicate significant superiority. The answer is tougher testing and more emphasis on hybrid pedigree. Inbred GCA in a pedigree is worth at least one year of testing."

Bill Landgren of Willmar, MN developed many useful early corn inbreds. He rarely developed two inbreds from different pedigrees exactly the same way. His source materials were often late elite inbreds on one side and various sources of earliness on the other. He used Howe's Alberta flint, HAW synthetic of early commercial hybrids made by Henry A. Wallace, and then moved on to some proprietary extremely early flowering inbreds from the Netherlands (see Figure 14.2, HT4). Landgren also recycled his own and other breeder's early inbreds. He grew observation blocks where he could personally study plants within a pedigree at flowering time to decide what to do next. He sometimes used a scheme we called alternate backcrossing, where he would backcross early segregates to the late parent inbred one summer generation and backcross late segregates to the early parent inbred the next. He would self or sib in the winter generation between backcrosses. Alternate backcrossing is an excellent way to break old linkages and form new ones with the selected traits of parent inbreds. Much of Landgren's corn breeding philosophy is in the source-sink selection scheme.

Source-sink selection scheme (Table 14.9) features selection both for earlier flowering, to increase grain-filling period (sink), and also for larger plant size, to increase photosynthetic area (source).[17] Resulting hybrids yield well both in warm or in long seasons, when photosynthesis is limiting, and also in cool or short seasons, when length of kernel-filling period is

limiting. As in the Rinke method, early- and late-maturity inbreds are used as source materials, and large populations (600 to 2000 plants) are grown at higher plant densities (twice normal) to select for early flowering. The source-sink scheme differs from the Rinke method by also including selection for more photosynthetic capacity (fast seedling and juvenile plant growth; tall, leafy mature plants; and stay-green). In total, source-sink scheme selects for productivity, early flowering, large plant size, disease resistance, stay-green, and longer kernel-filling period. Collectively these traits increase source and sink. I have omitted method B (no winter program) from Table 14.9.[18]

Selection for early flowering in late synthetics is used in the early corn growing areas to adapt and to improve populations.[4,18,86] Troyer and Brown advocated crossing exotic late germplasm with elite Corn Belt material, and then sibbing within populations for several generations to increase recombination. They practiced simple phenotypic recurrent selection for early flowering within large populations (>1000 plants) at higher plant density (twice normal) until local adaptation is attained. Results using ten different late synthetics showed selection for early flowering was effective, with an average reduction per synthetic of 9.5 days to flower, 7.5 moisture points less grain moisture, and an average yield increase per synthetic of 11% after six and seven cycles of selection. In the 2-year comparison, earlier flowering increased yield more in the shorter season than in the longer season.[4,18]

Faster rate of ear dry down is another way to make corn earlier because days RM is based on kernel moisture at harvest. There's a real advantage to keeping the plant alive yet have the ear dry rapidly.[18,76] Looser, thinner, and shorter husks better expose the ear to air movement. Thinner ears or multiple, smaller ears, and smaller kernels have more surface area per volume. Husks are modified leaves (leaf sheaths). Husks' thickness, width, and length are often similar to the leaves on the same plant. Plants with thinner, narrower, shorter leaves will have faster ear dry down. Shank internode pattern at the ear attachment end is similar to the stalk above the ear attachment node. Longer stalk internodes just above the ear indicate longer shank internodes near ear attachment — ear husks will loosen more. Condensed internodes at the ear attachment cause tight husks. Higher eared plants usually have fewer husks than lower eared plants because the stem above the ear and the shank develop nodes at a similar rate (see Section III J).

The ear is a solid cylinder with kernel caps forming the outer surface. Rough dent kernels provide more surface area for drying than do smooth, flinty kernels. Kernel size and shape affect rate of shelled corn drying — smaller kernels dry faster. Flinty kernels dry slowly both on the ear in the field and also after shelling in the drier — their endosperm is concentrated (hydrophilic) and equalizes with atmospheric humidity at higher kernel moisture than dent kernels.

H. Miscellaneous

Biotechnology has brought many bright, young, well-trained people to corn breeding, and biotechnology has greatly increased corn breeding budgets. This is a larger influx of people than for population geneticists, plant pathologists, mutation breeders, and plant physiologists in the past. Some of these new people will find a life-long vocation in corn breeding. It is good for corn breeding to absorb new disciplines.

Plant breeding uses biotechnology as a tool to increase its own efficiency, and to improve disease and insect resistance, or to add novel kinds of herbicide tolerance according to Duvick.[88] The relative importance of herbicide tolerance compared with yield increase over time is not yet known. Increasing emphasis is being placed on creating novel industrial and food products from plants. Advantages may include renewable resources, lower cost, being more biodegradable, or being novel. Hybrids with novel herbicide tolerance and insect resistance are being marketed. Commercial seed companies, chemical companies, and pharmaceutical companies are interested in producing novel chemicals from genetically altered plants for profit.[1]

TABLE 14.9
Four Versions of Source-Sink Selection Scheme

Year	A. Minimum Time	C. Late Population
One Summer	a. Grow 600 to 2000 plants b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.	a. Grow 600 to 2000 plants. b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.
One Winter	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each 10 to 15 vigorous, stay-green rows	a. Backcross a bulk of 100 plants to early parent.
Two Summer	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each 10 to 15 vigorous, stay-green rows	a. Grow 600 to 2000 plants. b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.
Two Winter	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each 10 to 15 vigorous, stay-green rows	a. Backcross a bulk of 100 plants to early parent.
Three Summer	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each 10 to 15 early, tall, stay-green rows	a. Grow 600 to 2000 plants. b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.
Three Winter	a. Make test crosses.	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each 10 to 15 vigorous, stay-green rows
Future	a. Grow test crosses. b. Use best inbred lines. c. Recycle best 8 to 10%.	a. Develop inbred lines and test. b. Use best inbred lines. c. Recycle best 8 to 10%.

Year	D. Early Population	E. Difficult Population
One Summer	a. Grow 600 to 2000 plants b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.	a. Grow 600 to 2000 plants. b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.
One Winter	a. Backcross a bulk of 100 plants to late parent.	a. Sib a bulk of 200 plants.
Two Summer	a. Grow 600 to 2000 plants b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.	a. Grow 600 to 2000 plants. b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.
Two Winter	a. Backcross a bulk of 100 plants to late parent.	a. Sib a bulk of 200 plants.
Three Summer	a. Grow 600 to 2000 plants b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.	a. Grow 600 to 2000 plants. b. Self earliest 50 to 100. c. Select 20 to 30 tall, disease resistant, stay-green, well-eared plants.
Three Winter	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each of 10 to 15 vigorous, stay-green rows.	a. Ear-row 20 to 30 ears. b. Self 6 per row. c. Save 2 or 3 ears from each 10 to 15 vigorous, stay-green rows.
Future	a. Develop inbred lines and test. b. Use best inbred lines. c. Recycle best 8 to 10%.	a. Develop inbred lines and test. b. Use best inbred lines. c. Recycle best 8 to 10%.

Molecular markers will provide more biological insights into plant breeding and genetics. Openshaw and Frascaroli summarize and comment on a number of encouraging studies of marker assisted selection (MAS) for quantitative trait loci (QTL) by others.[89] They then describe three rather large QTL detection experiments for grain yield, grain moisture, not stalk lodged, not root lodged, and plant height. Each experiment involved 250 F3 inbreds from an elite breeding population evaluated at multiple environments. They found zero to only five yield QTL, then predicted values for each breeding population, and completed three cycles of divergent selection for yield with little effect. They then designed a larger experiment hoping to benefit from their recent experience. In spite of a better breeding population, more and better selected plants, more and better markers, and yield trial data from 19 locations, they did not find QTL with extremely large effects for any of the previously mentioned agronomic traits. The largest QTL effect for yield that appeared to be segregating was only 188 kg ha^{-1}.[89]

Molecular marker assisted backcrossing is widely used to speed the backcrossing of herbicide tolerance and insect resistance genes into inbreds. It may also be useful for reducing undesirable, donor parent DNA. Getting rid of what you don't want is important. In mice with dense RFLP genetic maps, it ordinarily takes 20 to 30 generations to develop congenic (isogenic) mice strains with a selected genomic region.[90] They reduce number of generations by using markers to select against parents with chromosomes containing unwanted, donor parent DNA. Molecular markers can help us do a more complete, better job of backcrossing — not just a faster job.

Molecular genetics may be ultimately useful to sequence genes for a desired amino acid or protein to add to an inbred. It may become possible to delete unnecessary repetitive DNA, making the genome smaller and faster dividing thus speeding maturity. Perhaps deleterious recessive genes can be removed or masked to improve performance of inbreds in seed production fields. This would be very helpful.

Genome projects are unraveling the corn genome. Comparative genomics show many shared genes. Some shared genes have subtle differences that may be useful. Ultimately, genomics may result in the design, development, and manipulation of biologically and commercially important molecules. Efforts on gene expression and function are increasing. Expression and allele libraries will associate phenotypes with specific loci and alleles. The National Science Foundation awarded $85 million in grants for plant genome research in 1998.[1]

Sources of pest tolerance are likely to come from geographic areas where the pest of interest was a problem. Knowing the origin and history of materials (biogeography) often helps in choosing better breeding material. Open-pollinated varieties that coexisted with either a disease or an insect have undergone natural selection for tolerance. Potency of tolerance can be determined by growing segregating populations using a susceptible tester and noting frequency and degree of tolerance in segregates.[91] I have used Jenkin's method with proprietary materials, and it was useful.

Creating epiphytotics and infestations for selection is usually more efficient than traveling to where they exist naturally. Multiple inoculations and infestations increase the success rate. Mechanizing inoculation and infestation is usually cost effective. Late planting and early inoculation favor epiphytotics and infestations because seedlings are still succulent and tender when the pathogen or pest is more prevalent in the advanced season (see Section III C).

I. Breeding Superior Inbreds Summary

What is the nature of corn in your area and how can you improve it? How can you best exploit your breeding material(s) in your environment(s)? Select on a row basis. Phenotypic selection is effective. Multiple nursery observations enhance phenotypic selection. Select for traits associated with yield under stress that have higher heritability than yield itself. Phenotypic selection during inbreeding develops easier-to-produce hybrids. Early testing is expensive and limits population size; late testing is slow starting. Start with early testing and work your way into later testing. Cumulative selection with the FAST method is a good compromise. Genome conservation maintains favorable epistatic effects. Choose breeding methods based on objectives and on materials. More than one way to breed corn exists. Learn the pedigree method well. Efficient corn breeding programs use time and seed well.

Organized nurseries increase efficiency. PEPPER increases number of selection rows per dollar. Winter nurseries are at least half of your corn breeding life. Choose breeding materials based on unbiased research data (high GCA for yield and yield/moisture). Elite inbreds are the best source materials. Heterotic groups help in constructing breeding starts, and heterotic patterns help in choosing testers. Heterotic patterns are heritable. Biotechnology, genomics, and informatics are the future hope. Adaptedness is critical; quality of germplasm (better acclimated to their present environment) is more important for yield than quantity and diversity of germplasm (number of different chances). Corn breeding is *not* a numbers game. The game is to improve the adaptedness of a tropical crop to the temperate U.S. Corn Belt environment. Better adaptation to the environment means higher yield.

V. DEVELOPING SUPERIOR HYBRIDS

A. Background and History

Average U.S. corn yields by years over a 135-year period are shown in Figure 14.5. The U.S. average yield has surpassed 7839 kg ha^{-1} (125 bu/acre) in 4 of the past 5 years. Hybrid corn was made practical by the Jones double cross formula in the early 1920s.[92] During the 1930s the use of more hybrid corn, more mechanization, more fertilizer, and more plants per acre caused a yield explosion. Corn hybrids were more productive than the best varieties or synthetics. Superior stalk strength of hybrids allowed mechanical harvest. Hybrids removed the need to buy locally produced seed, thus allowing aggressive, research-oriented, seed-corn companies to increase in size.

Single-cross hybrids arose in the early 1960s after 40 years of improved corn hybrid technology. Better inbreds and better cultural practices made single-cross hybrids possible. These hybrids are faster to make and faster to produce (faster feedback). They are also easier to recognize (more uniform) and easier to breed (only two inbreds need be unrelated instead of three or four). The easier-to-recognize single-cross hybrids, plus combine harvest that provided quick, easy yield comparisons,

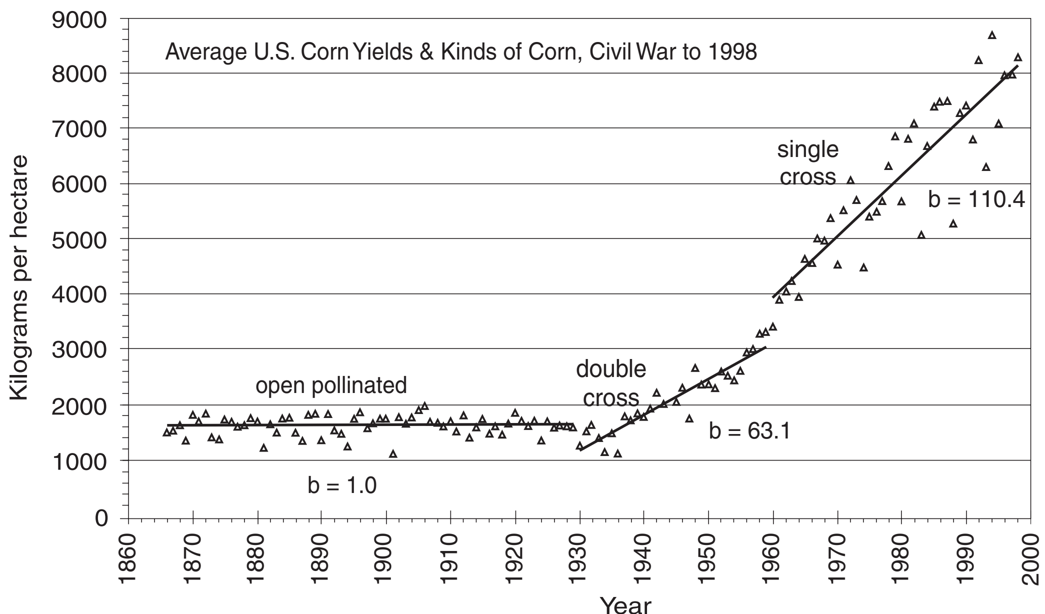


FIGURE 14.5 Average U.S. corn yields and kinds of corn, Civil War to 1998; periods dominated by open-pollinated varieties, by four-parent crosses, and by two-parent crosses are shown. ‘b’ values (regressions) indicate average gain per year. Data compiled by USDA.

caused better hybrid choice by the farmer. During the 1960s the use of more and better single-cross hybrids, more and cheaper nitrogen fertilizer, more and better herbicides, and still more plants per acre caused a second yield explosion. How much of the explosion is due to genetic gain?

Duvick studied yield genetic gain of U.S. hybrid corn for the 1930 to 1980 period.[93] He evaluated 47 commercial hybrids and an open-pollinated variety that were released from 1930 to 1978 in trials grown in 1978 and 1980. He found 89% of Iowa's estimated total yield gain over 50 years was due to genetic improvement. His second estimate based on single cross diallels for the same 50 years showed 71% genetic gain. Newer hybrids had better roots, better stalks, better stay-green, more resistance to barrenness, better resistance to second-generation European corn borer, and a more upright leaf habit. Flowering date, plant and ear height, grain moisture at harvest, and leaf area index changed little over successive eras. (However, combine harvest in the 1960s caused a shift to earlier flowering, shorter, lower-eared hybrids in a given area.)

Duvick summarized 13 studies of genetic gain that averaged 64% (Table 14.10).[94] He also listed typical changes that occur with genetic yield gains including: better harvest index, better stay-green, better stress tolerance (resist barrenness), better conformation to accommodate crowding, better conformation to accommodate harvesting, and better pest tolerance. Newer hybrids are better able to withstand the wide changes of too hot or too cold, drought or flood, disease or insects, higher plant densities or reduced stands, and too much or too little nitrogen. Improvements have been made for all these problems. Newer hybrids are tougher. If 64% of the increases of U.S. average yield are due to genetic gain, then 36% of the increases are due to improved cultural practices.

Genetic gain studies grow hybrids from different eras under the same cultural practices on fields that have received build-up applications of fertility. This reduces cultural practice effect. A modern hybrid grown on land that has never had improved cultural practices at the Morrow Plots, University of Illinois, averaged (1994–98) 30.1 q/ha. The same hybrid grown with 200 kg N per hectare, plus enough P and K to maintain soil test at 40 and 300, respectively, averaged (1994–98) 83.4 q/ha.[95] This shows 177% gain due to improved fertility alone. Improved cultural practices and corn production agronomists deserve more credit than they typically get in genetic gain studies. Two particularly good studies that partition increases in average yield of corn follow.

TABLE 14.10
Summary of Genetic Gains for Corn Grain Yield for Iowa

Author	Year Reported	Time Span	Experiment Years	Total Gain (kg/ha/year)	Genetic Gain (kg/ha/year)	Genetic Gain (%)
Frey	1971	1926 to 1968	1926 to 1968	—	—	56
Darrah	1973	1930 to 1970	1930 to 1970	0.99	0.33	33
Russell	1974	1930 to 1970	1971 to 1973	0.78	0.63	79
Russell	1974	1930 to 1970	1971 to 1973	0.78	0.49	63
Duvick	1977	1935 to 1971	1972 to 1973	0.88	0.50	57
Duvick	1977	1935 to 1972	1972 to 1973	0.88	0.53	60
Tapper	1983	1930 to 1970	1980 to 1981	—	—	42
Tapper	1983	1930 to 1970	1980 to 1981	—	—	67
Castelberry et al.[3]	1984	1930 to 1980	1980 to 1981	1.10	0.82	75
Duvick	1984	1930 to 1980	1978 to 1980	1.03	0.92	89
Duvick	1984	1930 to 1980	1977 to 1979	1.03	0.73	71
Russell	1984	1930 to 1980	1981 to 1982	0.90	0.71	79
Russell	1984	1930 to 1980	1981 to 1982	0.90	0.50	56
Average						64

a Estimate for the U.S.

Source: Duvick, D. N.[94]

Sundquist et al. made a technological assessment of U.S. commercial corn production that included analysis of nine major inputs.[96] In their thorough, comprehensive report they note the significant and persistent rise over time in planting rate (plant density) accompanied by new hybrids that yield well at higher plant densities. They determined a 22.5 q/ha increase over the 1954 to 1980 period from nitrogen versus a 16.9 q/ha increase from non-nitrogen technologies (including genetic gain). They state that little potential yield gain from additional nitrogen is likely in the future, that farmers are doing an adequate job of choosing hybrids, and they suggest that a change in emphasis by breeders could result in less chemical use (pesticides). They show nitrogen cost decreasing from nearly $200 per ton in the 1950s to almost $50 per ton in the early 1970s. They emphasize that virtually 100% of corn acreage was receiving herbicide treatment in 1980. They estimate loss due to disease at 10 to 14%, and to insects at 12%, with present control practices, and estimate losses due to weeds in absence of control can reach 100%. They show increases in reduced tillage from 18% in 1972 to 34% in 1980. Reduced tillage offers a short-run economic gain of about 10% of total labor and energy use per area of corn. However, the savings may be 50% for planting, weed control, and seedbed preparation in the spring when expenses are high and income is low. Minimum tillage conserves moisture. An emerging technology questionnaire was sent to 133 leading scientists with 110 returns. The returns showed expected yield increases from 1980 to 2000 for U.S. corn, including 10.0 q/ha from plant growth regulators, 5.0 q/ha from photosynthetic enhancement, 1.9 q/ha from cell and tissue culture, and 14.5 kg N per ha per year from biological nitrogen fixation. It is difficult to predict the future, and practitioners of new technologies are necessarily optimistic.

Cardwell analyzed the production practices affecting 50 years of increased average corn yields (20.0 to 62.5 q/ha) in Minnesota.[97] Change to hybrids produced 58% gain. Nitrogen net effect (adding nitrogen, subtracting reduced manure and other organic matter) produced 19% gain. Improved weed control produced 23% gain. Increased plant densities produced 21% gain. Earlier planting produced 8% gain. Drilling rather than hill-drop planting produced 8% gain. Fall rather than spring plowing produced 5% gain. And, narrower row spacing produced 4% gain. Second-year corn caused 7% loss, and root-worm damage caused an additional 3% loss. Corn borer caused 5% loss. Soil erosion caused 8% loss, and other unidentified negative factors caused 23% loss. He further reported that three-way hybrids had 2.6% superiority and single cross hybrids had 5% superiority over double-cross hybrids.

Large-scale nitrogen production, herbicide production, and insecticide production were all post-World War II phenomena. Nitrogen applied for U.S. corn increased very rapidly (approximately 2.3 kg/ha/year) from 1960 until 1980, then leveled off at about 130 kg/ha (130 lb/acre). Herbicide-treated fields for U.S. corn increased rapidly (approximately 4% per year) from 1950 until 1975, then leveled off at about 96% treated fields. Insecticide treated fields for U.S. corn increased (approximately 2% per year) from 1950 until 1970, then leveled off at about 40% treated fields.[71]

U.S. corn seeding rates have doubled in 40 years (approximately 2.5% per year). Corn breeders can effectively select materials that perform well at higher plant densities.[4,19] Late season stand counts increased 9.5% from 1994 to 1998 in Illinois.[98] The recommended plant density for corn in the central U.S. Corn Belt now exceeds 74,000 plants per hectare (29,600 per acre).[99] Increased seeding rate is a reason to expect U.S. average corn yield to continue to increase because of cultural practices. Extremely high wheat yields in Europe result from multiple nitrogen applications. Multiple nitrogen applications could further increase corn yields without causing nitrogen pollution of the environment.

B. Satisfy The Customer

Successful businesses satisfy customers. Seed corn business customers are seed corn dealers and farmers. Dealers want a reliable supply of competitive hybrids. What does the farmer want; how do you satisfy him? Value to the farmer is consistently high performance of the hybrid. Cost of

seed is a small input relative to other costs such as fuel, labor, fertilizer, pesticides, loan interest, and depreciation. The farmer expects to receive, and in fact does receive, a high return on investment for seed corn purchases. Because of this economic return for the farmer, price of seed has sufficient margin to fund intensive corn breeding research. It is a beautiful cycle that benefits all concerned.

A typical farmer plants six to eight hybrids. He probably has a main hybrid on 40 to 50% of his acreage that has performed well for him the last few years. He has two or three newer hybrids on 30 to 40% of his acreage, and has four or five brand new hybrids on the remaining 10 to 20%. The farmer has his own testing program and advancement system based on performance on his farm(s). How does he choose *new* hybrids? It is usually referral from his seed dealers, but it can be referral from his neighbors in the local coffee shop. It can also be from results of state performance trials or from results of local strip tests.

The farmer wants reliable hybrid performance that fits into his strategy for making profits. All farmers want more profit per unit area, but the hybrid that gives it will differ by geographic area due to maturity and disease(s), due to farming practices (tillage practices, irrigation, livestock production or cash grain, etc.), and due to time and place of marketing.

The most desired performance traits are consistently higher yield, lower kernel moisture, and easier harvest. Easier harvest includes good stalks, good roots, and good ear retention. Lower kernel moisture includes proper flowering time for the area and good ear dry down. Consistent, good performance over different years is desired. Drought tolerance and disease resistance may be desired. Many farmers see good emergence, early vigor, and ear retention as a must. They expect all hybrids to grow well and to hold their ears.

Farmers have long memories, but they are most impressed by the most recent season. They know weather is cyclical and is subject to warming and cooling trends over years. They often prepare for the coming season to be like last season.

Few variables affecting corn performance are fixed. Day-length pattern is the same for a given latitude. Soil type is fixed for a given portion of land, but virtually all farmers have multiple soil types on their farms. Even so, farmers plant later maturity corn on sandy river-bottom fields than on heavier, clay soils because sandy soil warms up sooner in the spring when lower temperatures limit corn growth. Johnsongrass is fixed. The farmer either has Johnsongrass or he doesn't. If he does, he probably has corn virus disease problems, and should probably buy a virus-tolerant corn. Season is relatively fixed. Season length and season GDU accumulation are constant enough that useful maturity zones for corn can be constructed. Nevertheless, seasons (years) are the major source of genotype-by-environment interaction for corn. Seasons have so many unusual weather happenings that they are seldom judged average until several years have passed that dull our memories of the weather details. Multiple year analyses of hybrid performance measure hybrid inconsistency due to seasonal effect.

Genotype by environment interaction is very important in corn. Corn differs from other major crops by having an imperfect flower. Because of the physical separation of male and female flowers, corn interacts with the weather more than other crops. The tassel is the male flower and the ear is the female flower. Female flowering is delayed by moisture stress (silk delay). Perfect-flowered crops, like wheat and oats, are more likely to interact with presence or absence of disease than with variation in the weather. Weather is the most important variable in corn performance (see Sections III A, III G).

Obviously, extreme heat or cold can kill corn tissue, but the most common major weather factor affecting corn is lack of water. Virtually all corn plants suffer from lack of water, most often at flowering, during their lifetime. Lack of water at flowering time is particularly critical because the silks are 90% water. Lack of water at flowering time slows or stops silk emergence. Tassels shed at the normal time but silks are delayed (silk delay). Evolution theory teaches that the fittest individual has the most surviving progeny. Silk delay decreases number of progeny by disrupting fertilization. The plant is less able to reproduce, which is the ultimate stress. Ability to tolerate higher levels of drought stress without silk delay signals tolerance to lesser stresses of other kinds

for corn. Selection for higher plant density stress tolerance has made the newer hybrids tougher (see Section V A).[19]

U.S. Corn Belt farmers understand how the weather is affecting their corn crop. Especially in midsummer when corn is flowering. Any conversation with a farmer that lasts more than a minute or two during the growing season in the U.S. Corn Belt will include his update on the local weather and an opinion on how it will affect his corn yield. Commodity traders are of the same mind. Weather markets develop rapidly with hot, dry weather in midsummer — especially following a dry year that decreased subsoil moisture.

C. Positioning Hybrids

Hybrids grown successfully north of their adapted maturity zone will usually have the following characteristics. A successful, late, full-season hybrid will flower relatively early for its RM. It will have moderate plant height for its RM, and have a longer kernel-filling period. It will have good yield for its RM, because higher yield will be expected of later full-season hybrids. It will have adequate test weight, because the kernel-filling period will be shorter. It will have adequate ear dry down, because the time period between physiological maturity of the kernel and harvest will be reduced. (see Sections I E, III G)

Hybrids grown successfully south of their maturity zone will usually have the following characteristics. A successful early, short-season hybrid will flower relatively late for its RM. It will have relatively tall plant height for its RM, to develop a larger plant to better compete for yield with later hybrids. It will have higher yield for its RM, because it will be compared to later, higher-yielding hybrids. It will have good stalk quality, good ear retention, and good stay-green, because it will be more physiologically mature at harvest than competing, adapted hybrids in environments that have more days and more GDUs from flowering to harvest. It will have adequate leaf disease tolerance, because leaf area (photosynthesis) will be limiting, and because the likelihood of leaf diseases is greater in longer-season areas on hybrids that are relatively older as measured from flowering (see Sections I E, III G).

Shorter and longer seasons are important variables in describing different years. Shorter seasons may be due to early frost or because of fewer GDUs accumulated during the season. Conversely, longer seasons may be due to more growing days available before frost or due to more GDUs per day that advance the development of the crop. Similar shorter- and longer-season effects occur in shorter- and longer-season maturity zones within years. Therefore, performance of a hybrid over shorter- and longer-season maturity zones within a year will help predict its performance consistency over years where season length differs.

To a lesser degree, but in a similar manner, north to south dispersion of test locations for a station provides more information per linear distance than east to west dispersion. Three maturity zones are usually a suitable testing area for a research station (see Figure 14.1). Of course, exchange hybrids will be tested in additional maturity zones by other research stations.

The Mississippi River is a convenient reference point for dividing east and west adaptation in the U.S. (see Table 14.11). Western adaptation requires more heat and drought tolerance. Both dry land and irrigated tests are needed in about the proportion existing in the area served. More dry land tests can be used for more stress tolerance emphasis. Western hybrids can get by with less ear rot and stalk rot disease tolerance because of less fall rainfall. Western hybrids have fewer test-weight problems, because corn is usually harvested at lower kernel moisture due to drier, windier fall weather. Western hybrids need better ear-retention. Eastern hybrids need more leaf, ear rot, root rot, and stalk rot disease tolerance because of more rainfall. Virus tolerant hybrids (MDMV and MCDV) are needed for areas near the Ohio River Valley, for Tennessee, and for other areas where Johnsongrass is present; CLN virus tolerance is needed in the Republican River Valley of Kansas and Nebraska.

The Alps are a convenient reference point for dividing east and west adaptation in Europe (Table 14.11). Eastern European hybrids require more heat and drought tolerance. Both dry land

TABLE 14.11
Rainfall and Temperature Data for Major Cities in Corn Growing Areas

	Elevation (m)	Rainfall[a] Annual (cm)	Rainfall[a] May–Sept (cm)	Temperature[a] Annual (°C)	Temperature[a] May–Sept (°C)
Near 45°N Parallel					
Minneapolis/St. Paul, MN (U.S.)	256	72.6	45.7	7.2	18.9
Montreal, Canada	36	103.6	44.2	5.8	17.4
Bordeaux, France	49	83.1	27.7	12.8	18.4
Venice, Italy	17	74.2	34.2	14.2	21.6
Krasnodar, USSR	33	65.0	23.2	10.8	19.4
Harbin, China	145	54.6	45.7	4.2	18.8
Near 40°N Parallel					
Denver, CO (U.S.)	1625	38.6	21.1	10.3	19.3
St. Joseph, MO (U.S.)	252	86.6	55.5	12.2	22.7
Springfield, IL (U.S.)	181	85.3	44.4	11.7	21.5
Indianapolis, IN (U.S.)	243	100.6	47.0	11.7	21.1
Columbus, OH (U.S.)	249	94.5	45.2	11.7	20.8
Philadelphia, PA (U.S.)	4	102.4	46.5	12.6	21.5
Toledo, Spain	607	40.9	11.4	13.9	21.0
Belgrade, Yugoslavia	101	64.8	30.5	11.9	19.7
Beijing, China	51	62.7	56.9	12.5	23.5

[a] Courtesy of Dr. Wayne M. Wendland, State Climatologist, Illinois State Water Survey, Urbana IL 61801.

and irrigated tests are needed. Eastern European hybrids can get by with less ear and stalk rot disease tolerance because of less rainfall. Western European hybrids need more ear and stalk rot disease tolerance because of more fall rainfall. Hybrids for the northern part of Western Europe need harder kernel texture (flinty kernels) for higher moisture combine harvest. Hybrids for Brittany, France need stronger roots to decrease root lodging due to frequent wind and rainstorms. Test weight problems and leaf disease problems are seldom serious in Europe; however, leaf disease problems will become serious in Europe if susceptible hybrids become popular.

D. Hybrid Stability

Eberhart and Russell stability regressions indicate whether a hybrid has an advantage at higher (regression >1.0) or at lower (regression <1.0) yield levels.[100] A regression of exactly 1.0 indicates that its relative position to other hybrids stays the same over a number of locations with different average yield levels. Hybrids that win state and national yield contests often have stability regressions greater that 1.0. The hearts, the minds, and the loyalty of farmers are often won with stability regressions of 1.0 or slightly less. The ideal stability regression from a breeder's viewpoint is 1.0 with a high intercept on the vertical (yield) axis (see Section IV E).

Ceccarelli discusses the contrasting philosophies of improving crop production within specific macro-environments compared to across specific macro-environments.[101] He emphasizes that morphological and physiological plant characteristics for maximum yield under optimum conditions are different from those associated with maximum yield under stresses. He generates variety crossover interactions with widely different (0.80 vs. 1.17) stability regressions extended to very low 18.2 q/ha (29 bu/acre) yield levels. He emphasizes these lower yield levels must be specifically bred for. Stability regressions for popular hybrids in the U.S. Corn Belt range from 0.95 to 1.05, and farmers expect more than 18.2 q/ha at planting (hybrid choice) time. However, the regression crossover principle applies. Regressions less than 1.0 are considered defensive and regressions

greater than 1.0 are considered offensive. Progressive farmers understand this concept and choose hybrids accordingly based on risk tolerance.

Ceccarelli advances a somewhat similar argument for need of different plant types for high input than for low input cultivation.[102] He claims we have missed the opportunity to exploit genetic differences at low input levels. He cites statistics claiming 60% of global agriculture is resource-poor and produces 15 to 20% of world food. He argues convincingly that lower input breeding is necessary for lower input areas. He advocates selection in the target environment under suboptimal conditions. I agree with his point of view. Using percentage of mean to equalize locations and years at 100 serves the purpose of giving lower yielding locations and years equal emphasis with higher yielding locations and years in data summaries (means). Hayes and Garber advocated using percentage of check hybrid that also does this 70 years ago.[103] It is still a good idea. I advocate historical trial results in dollars per unit area on date of harvest. This also increases lower yield emphasis because lower-yield seasons cause demand markets with higher prices. Using higher planting densities for testing experimental hybrids helps target the right density for the future commercial hybrid. Realizing trends for increasing plant density and nitrogen use existed, and increasing inputs accordingly, was a great help to me and to my employers.

E. End Use

End use and value added have become fashionable terms in the last decade. This is partly because of biotechnology. About 20% of U.S. corn is used for food, alcohol, and industrial uses (about 30% of this 20% becomes by-product animal feed, so final food, alcohol, and industrial use becomes 14%). Dry millers use white corn for flaking grits, brewers grits, snack grits, corn meals, and corn flours. Hybrids providing larger grits were first differentiated in 1977. This was needed because newer, higher yielding hybrids with more soft starch in the kernels had more breakage resulting in smaller grits. About 830,000 ha (2 million acres) are used for yellow food grade snacks and for white corn. Waxy cornstarch improves the uniformity, stability, and texture of several dry, canned, and frozen food products. Higher amylose is used in confectioneries as a stabilizer, in puddings as a thickener, in corrugating as an adhesive carrier, in films and package coatings, and in degradable loose fill. DuPont licensed Dr. Alexander's high oil materials at the University of Illinois in 1989. DuPont has licensed high-oil inbreds to more than 20 seed companies. The higher oil provides greater total energy and more of some limiting amino acids. Total U.S. high oil corn production doubled every year for the first 4 years, and has made substantial gains since. About 800,000 ha (2 million acres) of high oil corn are estimated for 1999.[1]

Corn's popularity for industrial use (especially alcohol and wet milling) depends on corn being the least expensive source of starch, which in turn depends on high-yielding, low-cost corn production. More specialty corns will undoubtedly be successful, but I will be pleasantly surprised at a rapid increase in corn for food, alcohol, and industrial use in the near future.

F. Modern Information Management

Modern information management for breeding superior hybrids involves the best use of field plot technique, hypothesis testing, and inference suggesting for product advancement. Much of the information in this section comes from the future section of "Statistical methods in seed corn product selection."[21] I hope you read the paper for more information.

Modern testing programs place minimum emphasis on precision at an individual location and maximum emphasis on precision across locations and years.[104] Maximum emphasis will be placed on prediction of performance in the infinite number of customer environments that will occur. The concept of wide-area testing will expand. Single replicates of experimental hybrids will be grown at a given location to optimize seed dispersion over the most locations. Entry lists will be prepared by location, rather than by experiment, again to optimize seed dispersion. Error estimation

and maturity comparison at a location will be confined to the analysis of a few repeated, commercial, and competitor checks at the location. These checks will be arranged in a systematic control design within and among tests.[105] Hybrid comparisons of most interest will be most efficiently accomplished by ordering the hybrids closer to each other in the field in a restricted-randomization design. The only replication of experimental hybrid entries at a location will be by confounding replicates with densities, dates of planting, fertility levels, or some other important cultural or environmental variable.

Locations chosen for testing will be based on an analysis of the environments likely to occur to the hybrids. Environments will be ranked in order of likelihood and locations will be chosen to maximize the correlation of present with future. Emphasis will shift from yield level as an indicator of location suitability to the likelihood that the location will occur in the future customer environment. Major environments (those most likely to occur) will be sampled more and weighted more than minor environments (those least likely to occur). All locations' data will be included in data summaries, because they will be from an environment that will likely occur again. No data will be excluded from summaries unless an obvious fault in data collection occurs. A very small share of the researcher's budget will be devoted to error control at a location. A very large share of budget will be devoted to maximizing the predictive value of the across-locations data.

Maximum focus will be given to across-locations and across-years summaries for the specified hybrid. Hypotheses will be developed that help to biologically explain genotype–environment interaction based on measures of environmental variables such as length of growing season, rainfall, temperature, disease incidence, and fertility. It will be possible for all comparisons to be made among any two hybrids that were tested at common locations. Presentation of data will make total use of graphics software. Predicted performance of new hybrids under the range of environments likely to be encountered will be graphically presented. Thus, hybrid by environment interaction will be highlighted. Emphasis will be given to presentation of data in a form that both company managers and customers will understand.

From a sampling standpoint, each year and each location should have equal weight. This can be achieved by putting yield results into percent mean where each location and each year averages 100. Historical data base selection index should be reported in dollars using local price and discounts on date of harvest. Cash grain farming has increased at the expense of diversified farming. Nearly all farmers grow corn for income dollars. Using historical dollars more fairly appraises hybrids that do well in low-yield, high-stress years when prices are higher in a demand market or in a weather-related market.

Large testing programs may have 500 or more advanced hybrids that are candidates for advancement to precommercial level where pilot samples are made. These are important decisions because pilot samples are costly. The critical performance comparisons for advanced hybrids are to existing commercial and competitor hybrids of that maturity and to each other. Five hundred hybrids require more than 100,000 comparisons two at a time. These head-to-head comparisons are particularly useful because they bring data from more locations together than would a summary where more hybrids are compared at a time. These head-to-heads are facilitated by large, flexible entry lists (150 to 200), and by interspersing advanced hybrids spatially in different experiments within a maturity zone.[20]

The idea is to order a number of comparisons — more comparisons as the hybrid draws nearer the market place. All advanced hybrids of a maturity spread need *not* be in all tests; they should be distributed among tests so they can also be compared to each other and to precommercial, commercial, and competitor hybrids. The correct priority order for hybrid comparisons is commercial vs. competitor, precommercial vs. commercial, and then advanced hybrid vs. commercial and competitor. The testing program and advancement system should serve this priority order. For example, restricted randomization can group entries of commercials and competitors in the center of the block, closer together, for more accurate comparisons to each other and to other hybrids.

Head-to-head comparisons maximize the number of locations where two hybrids are compared.[20] These comparisons are particularly useful in conjunction with wide-area testing to maximize the number of locations per data summary. Statistical inferences are made with *t* tests.[21] Locations should contain descriptors (maturity zone, state and county, date of planting, previous crop, plant density, amount of nitrogen applied, etc.) to enable comparisons of the two hybrids under different conditions in all or in different areas.

A head-to-head comparison with a well-known popular commercial hybrid involving many locations will show a student of commercial hybrids virtually everything one needs to know about performance for the potential commercialization of the other, newer hybrid. Not everyone is a student of commercial hybrids. Financial people, that have little or no training in agriculture let alone plant breeding, make important inputs to hybrid advancement. Their input is wanted and needed. How can performance results be put in a form to help these people? Everyone realizes that market leaders exist. The answer is comparisons of a newer hybrid to the market leaders in a series of head-to-head comparisons called a head-to-group comparison.[21]

Head-to-group comparisons bring together appropriate head-to-head comparisons of the new hybrid in question to each of a series of appropriate market leaders.[21] Head-to-group comparisons assess the need for a new product. Statistical inferences are made using the probability of a greater *t* for tests of significance. Usually the group is assembled from commercial and competitor hybrids that range plus or minus some specific interval of maturity (RM). Some judgment is necessary in deciding the minimum number of tests (10, 20, 50, more?) to enter a head-to-head in a head-to-group comparison.[21]

It is easy for a parent company to determine how you have done. A more pertinent and more difficult question is how are you going to do? The seed corn industry is mature in the sense that all farmers are buying hybrid seed corn — additional customers must stop buying another company's seed to buy more of your seed. Seed corn sales are performance driven.[106,107] Better value for the farmer comes from better performing hybrids. Relative product performance of two companies can be compared with a group-to-group comparison.

Group-to-group comparisons measure the relative product-line performance of two companies at a time. If the market share of each component hybrid can be determined, group-to-group comparisons with weighted averages can quantitatively predict relative company performance impact on the market. It is important in group-to-group comparisons to compare hybrids of a similar vintage, because newer hybrids are expected to be better in a business where genetic gain is being made.

G. The Efficient Testing Program

An efficient testing program uses time and seed wisely. Usually three to five progressive hybrid testing levels are useful. A four-level scheme might designate hybrids for testing as follows: Preliminary hybrids requires a minimum amount of seed (200 to 400 kernels) of many hybrids (1000 or more per station) to test in a preliminary test at a minimum number of locations (1 to 5). Exchange hybrids require more seed (7000 kernels) to retest in advanced tests at home and at other research station's test locations (40 to 100). A pilot hybrid is retested in advanced tests like an exchange hybrid while a pilot production of 1250 to 2500 kg (50 to 100 bushels) is made. Precommercial hybrids are retested like an exchange hybrid in advanced tests by the research department and also tested by the marketing department in strip tests of 0.125 ha (0.33 acre) by farmers along with appropriate commercial and competitor hybrids for comparison.

An efficient testing program finishes with relatively few precommercial hybrids (30 to 40) with greater number of seeds (1250 to 2500 kg) that are tested like an exchange hybrid by research (40 to 100 locations) and in strip tests by marketing personnel and by farmers (100 or more locations). Research tests better measure traits from counts or scores. Strip tests better measure yields under farmers' conditions. At least 300 total tests from 3 or more years are necessary to accurately predict

the future performance of a hybrid. Multiple year results of commercial and competitive hybrids are necessary to evaluate hybrid by year interaction (consistency over years) and to evaluate the track record of the advancement system. Almost anyone can determine a hybrid's past performance. It takes a good testing program and good corn breeders to identify hybrids with potentially good future performance. More tests over more years identify more genes for adaptedness.

An efficient testing program minimizes the number of kinds of tests. The right minimum number for research is two — preliminary and advanced. A preliminary test contains hybrids being yield tested for the first time. An advanced test contains superior hybrids selected from previous years' tests (both preliminary and advanced). The advantage of fewer, larger tests is more useful head-to-head comparisons. Exchange, pilot, precommercial, commercial, and competitor hybrids of the appropriate RM all go in the advanced test for a given maturity zone.

An efficient testing program has defined levels of testing which allows making additional hybrid seed for the next level of testing during testing at the present level. Hybrids can be kept at the exchange level an extra year when critical information is lacking, and hybrids can skip a level of advancement when performance and seed amount allow. The program should be flexible in this respect. Again, multiple year performance is most important in assessing future consistent performance of a hybrid. The correct priority order is years, locations, and replications. How many locations are needed for preliminary tests? One location with two plant densities is the minimum number. More than five locations of one replication are probably wasteful; the effort should go into testing more hybrids. It is easy to discard hybrids in preliminary tests, because usually half the hybrids will have a glaring fault. Of course, more tests provide more information for discarding. The contradiction in corn hybrid selection is the ease of discarding inferior hybrids vs. the difficulty of identifying superior hybrids. The difficulty lies in hybrid by environment interaction.

Hybrids can be positioned among test locations by maturity zones to gain more useful information. About 50% of a hybrid's test locations should be located in the same maturity zone as the hybrid's RM, which is likely to be where the hybrid will be most sold. About 20% of a hybrid's test locations should be located in the next shorter-season maturity zone than the hybrid's RM to determine its performance as a late, full-season hybrid. And, about 30% of a hybrid's test locations should be located in the next two longer-season maturity zones than the hybrid's RM to determine its performance as an early, less-than-full-season hybrid.

Research plot size is usually 1/1235 ha (1/500 acre) in two-row plots. Number of plants per row (1/1000 acre) equals thousands of plants per acre. This area should include the alley to give a true estimate of yield and of plant density for the entire field (end border % = alley length/plot length × 100). Smaller plot sizes mean less soil variability and less shelled corn to handle. Marketing department strip test plot size is usually about 1/8 ha (1/3 acre). Strips are usually 6 or 8 rows wide. These larger, wider plots show less border effect among hybrids. A six-row plot has 1/6 the border effect of a one-row plot. Side border % = (1/number of rows) × 100. Strip tests have the advantage of being cared for by the farmer in a manner like the rest of the corn he grows. Both strip tests and farmers' fields have *no* alleys. Strip test results truly reflect farmers' cultural practices and planting pattern, and for this reason strip test results are most useful to everyone concerned.

Plant density has increased constantly over time. It takes time to identify and to position hybrids in the market, so "normal-density" yield-tests should be 10 to 15% above the average of progressive farmers in the area where each yield test is grown. "High-plant-density" yield-tests that induce moisture stress and self-shading increase broken stalks, barrenness, and dropped ears. They should be 25 to 30% above the plant densities of local, progressive farmers.[19] Alley width between ranges of plots should be as narrow as possible (<76 cm), because farmers' fields do *not* have alleys. Alleys are an artifact and their effect must be minimized. Plant density and yield per area calculations should include the alley to give whole-field estimates of plant density and of yield per area — like the farmer.

Time of planting and time of harvesting affect yield test results. They should be comparable to progressive farmers in the area. Early planting helps to better evaluate early stands and seedling

vigor (see Section III B) Timely harvest gives a better, more appropriate range of kernel moisture at harvest to determine relative maturity. Later harvest helps to better evaluate stay-green, broken stalks, and dropped ears. As more plants and more nitrogen per unit area are applied, more broken stalks are likely to occur. It is easier to evaluate broken stalks now than it used to be. However, if only a few broken stalks have occurred at harvest time, the plants should be pushed (uniform, horizontal pressure applied at the ear attachment node) to induce breakage. I prefer pushing one row of a two-row plot, then counting broken stalks in both rows. I try to attain 10% broken stalks average for the entire test plot area. Stalks can be pushed by hand or by mechanical means.

Border effect among hybrids has become more of a problem with narrower rows and higher plant densities. A substantial part of the effect is usually due to plant height. Taller plants have an advantage in small (narrow) plots when light is limiting (usually high-yield locations), and have a disadvantage when water is limiting (usually low-yield locations). If adjustments for plant height to yield are made by regression, they should be calculated on a location by location basis because plant height effect interacts with location environments. Another strategy is to compare yields only among hybrids of similar plant height from randomized tests. Early corn is source-limited, so taller hybrids are expected to yield more.

A recent change is to more four-row plots for research testing. Only the center two rows are harvested to avoid side border effect. Results from four row plots are necessarily confounded with the environments where they are grown. Decisions to use four-row plots were probably *not* made by efficient research station managers. I used three-row plots one year. They were much more expensive — more seed, more packets, more soil variability, more discard corn to handle, and more categories of testing that complicates priorities for seed use. Four-row plots are inefficient. In the future, yield monitors will estimate yield from the middle of the rows — no alley effect.

A commercial corn breeder's goal is to provide hybrids that farmers want to buy.

H. Modern Hybrid Development

The hybrid development strategy entails a comparison of hybrids in a broad range of environments using modern cultural practices to mimic farmers' hybrid selection processes. Tens of thousands of hybrids are tested at hundreds of locations every year. Preliminary tests by research personnel typically involve 1/1235 ha (1/500 acre) replicated plots in which up to 20 agronomic traits and up to eight diseases and insect pests are measured. Final tests by marketing personnel and farmers typically involve 1/8 ha (1/3 acre) strip test plots in which five or six traits are measured. Strip tests are planted by, cared for, and harvested by farmers using the same cultural practices that they use with the rest of their corn. The goal is to identify new, widely adapted hybrids that can increase farmers' net profit with consistent higher yield, lower kernel moisture at harvest, or easier harvest than current popular hybrids permit. For a maturity, it is a winner-take-all game, because farmers want the most profitable hybrid.[21,55]

Modern hybrids come from modern testing programs. How trustworthy is the testing program that provides information for product release? Product performance is critical to increasing seed corn market share.[99,100] Seed corn companies are reputable and well intentioned. Their future depends on repeat business. Decisions on product release are meant to please the customer. Poor products are only released by mistake. The seed-corn business is dynamic. Change has been the rule (see Section V A). Modern hybrid testing programs are frequently updated to stay modern.[21] Farmers continue to want the most profit, but the strategy for achieving it changes.

Again, a corn performance-testing program should be designed to mimic the farmer's hybrid selection process. It is important that artifacts (manmade artificial effects) in the testing program be eliminated or greatly reduced so results apply directly to the farmer's situation.

Many test sites (locations) in a testing program are important in sampling the total environment. Some performance traits are less likely to occur. Barrenness, broken stalks, root lodging, and dropped ears may be common some seasons and very important at least in some areas, but rare in

other seasons. Use of many locations provides a better sample of discerning environments. Rainfall distribution and subsoil moisture at flowering time that affect yields are better sampled. Weather is variable. Where I come from, there's a time-worn adage that only fools and strangers predict the weather. In the Midwest, weather forecasting during a drought is seen as a source of humor — the comic relief portion of the evening news. U.S. Corn Belt rainfall depends largely on thunderstorms that are a hit-and-miss thing. Prolonged heat and drought adversely affect irrigated corn because of increased evapotranspiration.

Wide-area testing is successful because it identifies genes for adaptedness. These genes are sensitive to longer day lengths, to cooler minimum temperatures, to drought, and to shorter season length for temperate area hybrids because domesticated corn originated in the tropics. Genes for adaptedness are also sensitive to moisture stress during flowering that disrupts fertilization (silk delay) because corn has an imperfect flower. Allard has reported the survival of 18 ubiquitous and frequent alleles present in all 94 Mexican races that survived into the 30 most popular U.S. public inbreds and also survived into the six inbreds most used as parents of U.S. public single-cross hybrids. He concludes that frequent (predominant) alleles contributed to adaptedness in many habitats and survived many cycles of selection.[17]

Widely adapted hybrids perform well under variable weather conditions. Variable weather knows no boundaries. Ohio has a higher average annual rainfall than Nebraska, but dry seasons like those occurring in Nebraska also occur in Ohio. Widely adapted hybrids are good for the farmer because they are more likely to consistently yield well. The farmer has less reason to be concerned that unusual weather will greatly reduce yield. Widely adapted hybrids are also good for the seed company because the marketplace can be serviced with fewer hybrids. Regional hybrids are regional because of a deficiency; they lack something that limits their adaptation. The number of seed corn companies headquartered in Iowa has decreased from 400 in 1940 to 100 in 1957,[108] to 40 in 1990,[109] to 28 in 1997.[110] If local adaptation were of much importance, more small seed corn companies would have survived.

Some research programs grow tests where diseases are likely to occur. They evaluate entries for disease with other performance traits at those locations. Advocates of this approach prefer naturally occurring epiphytotics that penalize yield and other traits of susceptible hybrids in the test fields. It allows real estimates of disease effects on performance. Another approach is to grow tests under artificially induced epiphytotics for disease evaluation only. These disease results, from separate tests, are listed separately, thus less convenient to use, and susceptible hybrids are not necessarily penalized for yield. Either approach will work. I personally prefer the former, if the scope of the testing program permits, because it is more natural and easier to use.

How many testing sites are necessary? Think big, because more is better. The goal is to adequately sample the environment of the geographic area where the company sells seed corn. More locations mean fewer surprises in future performance. Avoid rigid matrices that require an Act of Congress to modify. Make it easy to add locations. A location is only a sampling point. From a sampling standpoint, the results from each location should receive equal weight; otherwise, the locations with higher yields will determine the winners. Equal weight of locations can be achieved by putting results in percentage of location mean so that each location averages 100 for each trait.

Years vary; our only substitute for years is more locations. We are competing for genetic gain, which has a time value like money. We cannot afford to test forever to learn what we should have done after it is too late. What to do? This is a risk assessment problem. We need to consider what we do *not* know, then assess how much our ignorance can hurt us. Categorizing tested environments is the first step. Identifying total environments and their frequency is the second. Estimating economic values is third. Remember, relative performance counts. It doesn't hurt as much if it happens to everyone, but our goal is to be better than everyone else. Patterson et al. present an excellent historical perspective for establishing research goals for improvement based on risk assessment.[111]

The fiscal year for a seed corn business is typically September 1 through August 31. In the Northern Hemisphere, corn-research budget year is logically calendar year, January 1 through December 31. Last year's business should be done by December 31. Next year's business starts January 1. I am recommending that company hybrid advancement meetings be held in December.

Company hybrid advancement meetings should have representatives of all departments present. All departments should have a formal function. Research, marketing, and sales will have the largest input, and will lead parts of the meeting, but all departments will participate. The meeting will start with economist's estimates of next year's corn acreage. This will limit our total seed production. A brief competitive analysis is helpful. What's happening? Why? Commercial and competitor hybrids are reviewed in head-to-group comparisons. Then, precommercial and pilot hybrids are compared with commercial and competitor hybrids, usually in head-to-head comparisons to see if they have a place. For each hybrid, production provides numbers for ease and cost of production; foundation seed provides parent inventory available. Usually hybrids are discussed within two or three broad maturity groups. Data books of critical comparisons are prepared in advance. Twenty-five years ago I prepared a "paperless" advancement meeting where comparisons were projected on a screen. It bombed. Everyone wanted to underline or highlight data and write notes in the margins. The books were essential at that time.

The company goal is to provide hybrids that farmers want to buy.

I. Time is the Greatest Innovator

"And he that will not apply new remedies, must expect new evils, for time is the greatest innovator," is a quote by Francis Bacon from "Of Innovations" in *Bacon's Essays*. Certainly you can depend on change in corn breeding. It is a dynamic vocation.

Time favors the evolution of new pests. I heard Dr. Arthur. L. Hooker state that "the next disease problem in corn will be caused by a disease that the seed corn industry is presently ignoring." The historical record in the U.S. Corn Belt on southern corn leaf blight, on yellow leaf spot, on eyespot, and on gray leaf spot supports Dr. Hooker's statement. The same principle applies to insects and to weeds that become tolerant to pesticides. Murphy's Law has some application here. "What can go wrong will go wrong" if we let it because of natural selection and evolution occurring on pests in a monoculture crop system.

Time is the greatest innovator, and time is the final limitation. We each have an obligation to do our best and to complete all the cycles of selection that we can. F. D. Richey finished his corn-breeding career at the Tennessee Experiment Station. He usually ended his nursery tour talking about inbreds he had developed. He listed positive traits for each inbred, then its main fault. Because the faults differed among inbreds, he would suggest that another cycle of pedigree selection would cure some of the faults. Then F. D. Richey would lament, "but I have run out of time."

J. Developing Superior Hybrids Summary

The question of questions is how to increase seed corn market share. The answer is simple. One must provide better-performing hybrids relative to the rest of the industry. Accomplishing this is more complicated. One must improve the yield testing and hybrid advancement program, and also improve the inbred development program. Add more yield test locations. Do not discard lower yielding tests; give them equal weight by putting results in percent of mean. Use higher plant densities for inbred and hybrid selection. Use only two kinds of research tests — preliminary and advanced. More entries per test provide more head-to-head comparisons. Calculate multiple-year yield test results. Calculate multiple-year inbred GCA values for choosing breeding starts and for advancing hybrids. Use multiple-year comparisons, most years first, to advance hybrids. More meaningful information comes from more years and more locations. Encourage informal exchange of hybrids in advanced tests among breeders. This will complement the formal hybrid exchange

by an administrator. Budget money for programming software for research. Establish a research users committee to set priorities for research programming.

Your job is to increase genetic gain for desired traits, including consistently higher yield, lower kernel moisture, and easier harvest. You will succeed by better planning and by better execution than your competition. Cultural practices and weather greatly affect corn performance; many testing sites are necessary to adequately sample the total environment. Years are the most important cause of hybrid by environment interaction. Differing moisture availability at flowering is the most important cause of hybrid by location interaction in the Midwest. Additional tests identify more genes for adaptedness. Temperate corn most often lacks adaptation to longer day length, to cooler minimum temperature, to drought, and to shorter season length because of corn's tropical domestication. High plant density stress is the ultimate stress for corn. Selection of inbreds and hybrids under high plant density stress develops tougher inbreds and hybrids. Widely adapted hybrids perform well under variable weather conditions. Widely adapted hybrids are good for the farmer and good for the seedsman. Multiple-year results of commercial and competitor hybrids measure both consistency of performance and also value of the hybrid advancement system. Modern hybrids come from modern testing programs (eliminate artifacts) and from modern information management (pertinent comparisons). Strip tests mimic farmers' fields. Time is the greatest innovator; corn breeding is dynamic. When you are through changing, you are through.

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